BIOLOGICAL EVALUATION OF THE REVISED LOUISIANA WATER QUALITY STANDARDS

DO Criteria Revisions for Eastern Lower Mississippi River Alluvial Plains Ecoregion (LAC 33:IX:1123) (Rule WQ091)

PREPARED FOR:
U.S. FISH AND WILDLIFE SERVICE

PREPARED BY:
U.S. ENVIRONMENTAL PROTECTION AGENCY
REGION 6, ECOSYSTEMS PROTECTION BRANCH
1445 ROSS AVENUE
DALLAS, TX 75202

October 5, 2017

Table of Contents

1.0 Background Information

1.1 Consultation History

The Louisiana Department of Environmental Quality (LDEQ) completed a triennial review of the state's water quality standards at LAC 33:IX Chapter 11 of the Louisiana Surface Water Quality Standards on December 20, 2015 and submitted the revised standards to the U.S. Environmental Protection Agency (EPA), Region 6 on January 15, 2016. The EPA approved these amendments on June 3, 2016. In its action, EPA noted that its approval may be subject to the results of consultation with the U.S. Fish and Wildlife Service (USFWS) and the National Marine Fisheries Service (NMFS) pursuant to Section 7(a)(2) of the Endangered Species Act (ESA). EPA retains authority to take additional action regarding Louisiana's recently revised criteria if consultation identifies deficiencies in those criteria.

The EPA initiated informal consultation under Section 7(a)(2) of the ESA with the USFWS and the NMFS regarding EPA's approval of amendments to Louisiana's water quality standards through an initial February 2, 2017 email to USFWS Louisiana Ecological Services Field Office Deputy Field Supervisor, Brad Rieck. The EPA followed that email with a February 27, 2017 letter to Joseph Ranson, Field Supervisor. The EPA followed that communication with a letter to David Bernhart, Assistant Regional Administrator, NMFS. In those communications, EPA noted that Louisiana had adopted amendments to its water quality standards (WQS) at LAC 33: IX. 1123 and requested any information or input concerning possible effects of the amended dissolved oxygen (DO) criterion on threatened and endangered species in the affected area. The letters initiating consultation and those received from the Services in response to this request are provided in Appendix A.

The EPA defined the action area covered by the WQS amendments in the USFWS's Information for Planning and Consultation (IPaC) site. The IPaC site generated a species list. Although they have since deferred to USFWS on this consultation, NMFS also provided EPA with a species list through its Public Consultation and Tracking System (PCTS). The species list is also included in Appendix B.

1.2 Overview of Water Quality Standards and Criteria

Section 303(c) of the CWA requires that all states adopt water quality standards and that EPA review these standards. Every 3 years, the states are also required to go through a public process, commonly referred to as the triennial review, where the state reviews its water quality standards and, as appropriate, modifies and adopts new standards. This process allows states to incorporate new technical and scientific data into their standards. The regulatory requirements governing water quality standards are established at 40 Code of Federal Regulations (CFR) Part 131. The minimum requirements that must be included in the state standards are designated uses, criteria to protect the uses, and an antidegradation policy to protect existing uses, high-quality waters, and waters designated as Outstanding National Resource Waters. In addition to these elements, the regulations allow for states to adopt discretionary policies such as allowances for mixing zones and variances from water quality standards. These policies are also subject to EPA review and approval.

A water quality standard defines the water quality goals for a waterbody by designating the use or uses to be made of the water (“designated uses”), by setting criteria necessary to protect the uses (“criteria”), or by preventing or limiting degradation of water quality through antidegradation provisions (“antidegradation policy”). Thus, a state’s water quality standards consist of designated uses, water quality criteria, and an antidegradation policy. The Clean Water Act (CWA) provides the statutory basis for the water quality standards program and defines broad water quality goals. For example, Section 101(a) states, in part, a goal that wherever attainable, waters achieve a level of quality that provides for the protection and propagation of fish, shellfish, and wildlife, and for recreation in and on the water ("fishable/swimmable”).

The EPA publishes recommended criteria documents as guidance to states. States consider these recommended criteria documents, along with the most recent scientific information, when adopting regulatory criteria. All standards officially adopted by each state are submitted to EPA for review and approval or disapproval. The EPA reviews the standards to determine whether the analyses performed are adequate and evaluates whether the designated uses are appropriate and the criteria are protective of those uses. The EPA makes a determination as to whether the standards meet the requirements of the CWA and EPA's water quality standards regulations. The EPA then formally notifies the state of these results. If EPA determines that any such revised or new water quality standard is not consistent with the applicable requirements of the CWA, EPA is required to disapprove these changes to meet the requirements. The state is then given an opportunity to make appropriate changes. If the state does not adopt the required changes, EPA must promulgate federal regulations to replace those disapproved portions.

Section 303(c) of the CWA requires states and authorized tribes to adopt water quality criteria that protect designated uses. States and authorized tribes have four options when adopting water quality criteria for which EPA has published nationally recommended criteria pursuant to Section 304(a) of the CWA. States may: (1) adopt nationally recommended criteria; (2) adopt nationally recommended criteria modified to reflect site-specific conditions; (3) adopt criteria derived using other scientifically defensible methods; or (4) establish narrative criteria where numeric criteria cannot be determined or to supplement numerical criteria (40 CFR 131.11).

The nationally recommended criteria published in *Quality Criteria for Water*, EPA 440/5-86-001, commonly referred to as The Gold Book (USEPA 1986) recommends 5 mg/L as a one-day minimum for early life stages for warm water fishes. Except where site-specific DO criteria have been developed, the applicable DO criterion year-round for supporting the fish and wildlife propagation use is a minimum of 5 mg/L in fresh and marine waters, and a minimum of 4 mg/L in estuaries (LAC 33:IX.1113.C and 1123, Table 3). However, with these criteria in place, streams in Louisiana have been identified as impaired on the state’s CWA Section 303(d) list and identified for Total Maximum Daily Load (TMDL).

Since the 1980s, Louisiana has carried out site-specific studies that have documented that although many of its waters do not meet the present statewide 5 mg/L criterion (either on a daily basis and/or on a seasonal basis), these waters support fish and wildlife propagation uses. Inaccurate water quality criteria have resulted in erroneous use impairment decisions that impact many of the state's water quality programs. In response, LDEQ developed an ecoregion assessment approach in an effort to establish appropriate and protective DO criteria that support fish and wildlife propagation, and this approach has been used in revising the DO criteria for the western LMRAP (Barataria-Terrebonne basin) and eLMRAP that are the subject of this evaluation.

2.0 EPA Action

2.1 The Amended Louisiana Water Quality Standards and EPA Action

The federal action that is the subject of this biological evaluation is EPA's approval of amendments to the Louisiana Administrative Code (LAC) Title 33: Part IX, Chapter 11 Surface Water Quality Standards, Table 3 (WQ091):

These amendments establish a site-specific revised DO criterion. Specifically, the amendments establish a DO criterion of 2.3 mg/L between the months of March to November for 31 inland freshwater and estuarine stream subsegments in the eastern Lower Mississippi River Alluvial Plains Ecoregion (eLMRAP). The revised DO criterion applies to the following subsegments that are reflected in Table 3 in LAC 33: IX. 1123: 040201, 040303, 040305, 040306, 040401, 040402, 040403, 040404, 040503, 040506, 040508, 040601, 040604, 040605, 040606, 040702, 040705, 040809, 040907, 040915, 040916, 040917, 041101, 041201, 041202, 040807, 040808, 040903, 040912, 040913, and 040914.

No changes were made for waters in the eLMRAP between the months of December through February; a minimum criterion of 5.0 mg/L in inland areas and 4.0 mg/L in estuarine areas continues to apply except where site-specific criteria have previously been established.

The analysis of the effects of the approval of the revised DO criterion assumes that ESA-listed species and their habitat are exposed to waters meeting the revised water quality standards. The federal action under consideration at this time is whether EPA's approval of the revised standards will have an effect on the species of interest.

There are no direct effects to proposed or listed species as a result of EPA's approval of Louisiana's revised DO criteria in the eLMRAP. Approving new water quality standards in and of itself will not change the environmental baseline or directly affect listed species or species proposed for listing. However, there may be indirect effects of approving the revised DO criterion, because the approval allows implementation of the revised DO criteria. This includes NPDES permits, 303(d) assessment and listings, development of TMDLs, and water quality management plans designed to meet the standards over time.

2.2 Louisiana's Ecoregional Approach to DO Criteria

Louisiana investigated the use of an ecoregion approach to establish DO criteria for several types (i.e., streams, lakes, bays, canals, etc.) of waters (LDEQ 1996, DeWalt 1995, and DeWalt 1997). This ecoregion-based approach is intended to streamline the site-specific criteria derivation process by establishing a set of protocols that could be used on a routine basis to determine appropriate DO criteria for these categories of waters.

Through a 2008 Memorandum of Agreement (MOA), LDEQ and EPA agreed upon a protocol for determining the DO concentrations needed for protection of the fish and wildlife propagation use in Louisiana freshwater and estuarine streams, bayous, rivers, and lakes (LDEQ 2008a). This protocol uses an ecoregion approach to revise the DO criteria. The use of the ecoregion approach for revising DO criteria is intended to characterize water quality at reference (least-impacted) sites and allow for appropriate DO criteria to be determined for waterbody types or classifications within an ecoregion.

LDEQ refined statewide DO criteria descriptions, and adopted ecoregion-based DO criteria for the Barataria and Terrebonne Basins as presented in the *Use Attainability Analysis of Barataria and Terrebonne Basins for Revision of DO Water Quality Criteria* (2008). By definition, this document is not a Use Attainability Analysis (USEPA 1983); however, the assessment is referred to as a use attainability analysis (UAA) and was used to inform the development of ecoregional-based criteria in the Barataria and Terrebonne Basins. The Barataria-Terrebonne Basin UAA (BTUAA) supported criteria changes in 60 subsegments, with changes in 20 rivers and streams subsegments, to a minimum criterion of 2.3 mg/L during the critical season (i.e., March through November) (LAC 33IX.1123) (WQ091). Due to resource limitations, LDEQ was not able to include any data from the LMRAP east of the Mississippi River in the study; therefore, the statewide DO criteria of 5 mg/L (inland) and 4 mg/L (estuarine) remained applicable in this area of the state until 2016, except where site-specific criteria have been established (LAC 33:IX.1123.Table 3).

The ecoregion approach used in the BTUAA served as the basis for the DO criterion developed for the eastern LMRAP. The EPA approved the revised criteria for the Barataria and Terrebonne basins in May 2009. The EPA determined that there were no threatened/endangered species or critical habitat in the Barataria-Terrebonne basin, thus the approval of ecoregion-based DO criteria for the basin would have no effect on federally-listed threatened and endangered species or critical habitat.

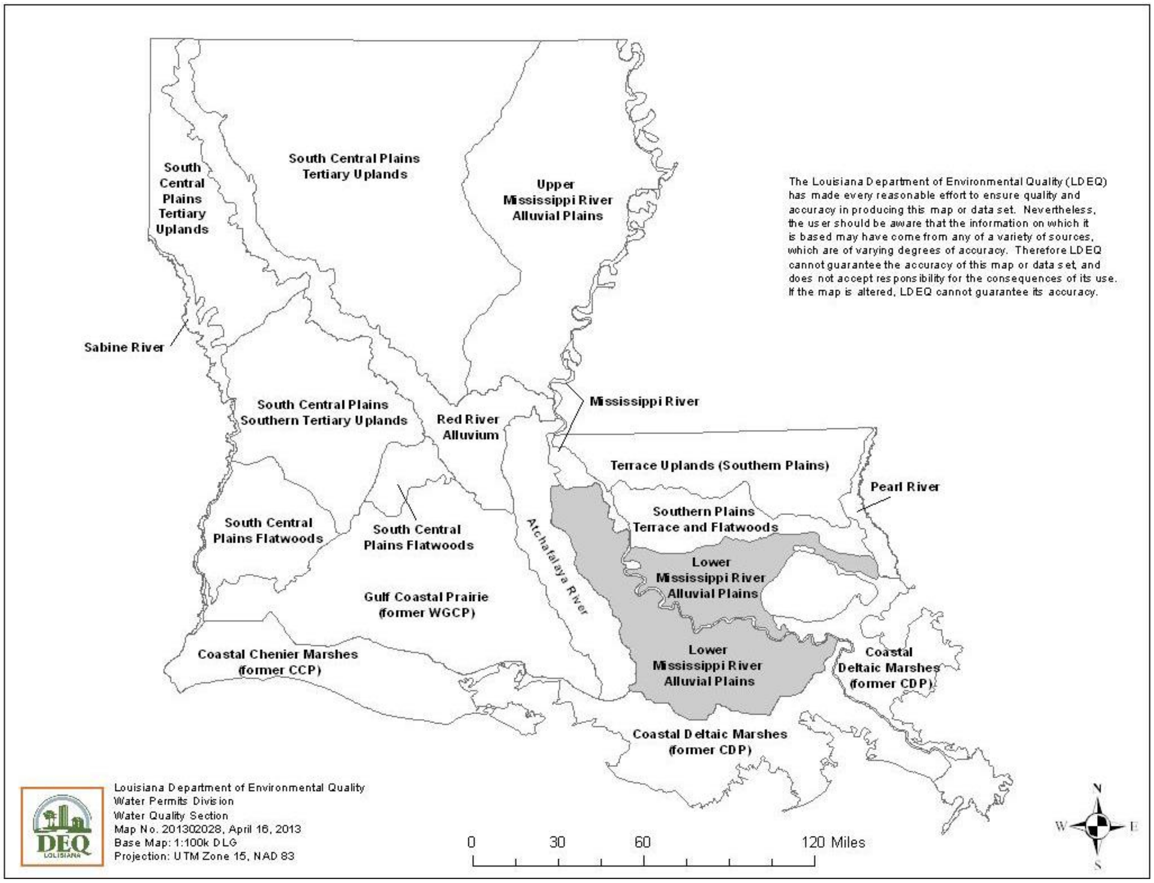
2.3 Basis for the Eastern LMRAP DO Criteria

The development of appropriate DO criteria depends on a well-developed assessment of the criteria necessary to support the designated uses. In this instance, the assessment included defining an appropriate study area and reference sites, chemical/physical/biological data collection, analysis, critical period determination and use/criteria derivation. As noted above, the findings in the Barataria and Terrebonne basins informed the work in the eastern LMRAP. The work in the eastern LMRAP was presented in the document: *Use Attainability Analysis of Inland Rivers and Streams in the Eastern Lower Mississippi River Alluvial Plains Ecoregion for Review of DO Water Quality Criteria* (2013).

2.3.1 Study Area

The eastern LMRAP UAA is a continuation of the process which began with the 2008 MOA and the BTUAA, and demonstrated that the revised DO criteria established for streams in the western portion of the LMRAP are also appropriate for the eastern portion of the LMRAP. The LMRAP ecoregion is bisected by the Mississippi River; the portion located to the west of the Mississippi River (i.e., the western "subecoregion") was addressed in the BTUAA, while the portion located to the east of the Mississippi River is the eastern LMRAP, as shown in **Figure 1**.

Figure 1. Water Quality Standards Ecoregions for Louisiana. Delineations include BTUAA and 2013 refinements for the eastern LMRAP.



2.3.2 Study Sites

The objectives of the eastern LMRAP UAA were to demonstrate the ecological similarity or dissimilarity between eastern and western portions of the LMRAP, establish appropriate critical and non-critical periods, and provide specific DO criteria recommendations for the eastern LMRAP.

2.4 Data Collection

Data collection efforts for the refinement of DO criteria in streams began in the western LMRAP in 2005 with the BTUAA. Several waterbody types (i.e., streams, lakes, canals, and bays) and the Coastal Deltaic Marshes (CDM) ecoregion were also included. There were 26 least-impacted sites sampled between 2005 and 2008, with eight of these stream sites in the western LMRAP subecoregion. In 2010, sampling began in the eastern LMRAP at six least-impacted sites (Table 1) and continued in the eastern CDM subecoregions using a similar monitoring design, with limited *in situ* water quality sampling in the western subecoregion. Sampling was interrupted in April 2010 by the oil spill in the Gulf of Mexico. In 2012, LDEQ collected additional continuous monitoring DO, pH, and temperature data and completed habitat assessments and fish sampling.

All reference sites in the western and eastern LMRAP were considered “least-impacted” by anthropogenic influences relative to the characteristics of the ecoregion based on a qualitative assessment.

Figure 2. Least-impacted sites and land use in the LMRAP ecoregion. Land use based on USGS data from 1998.

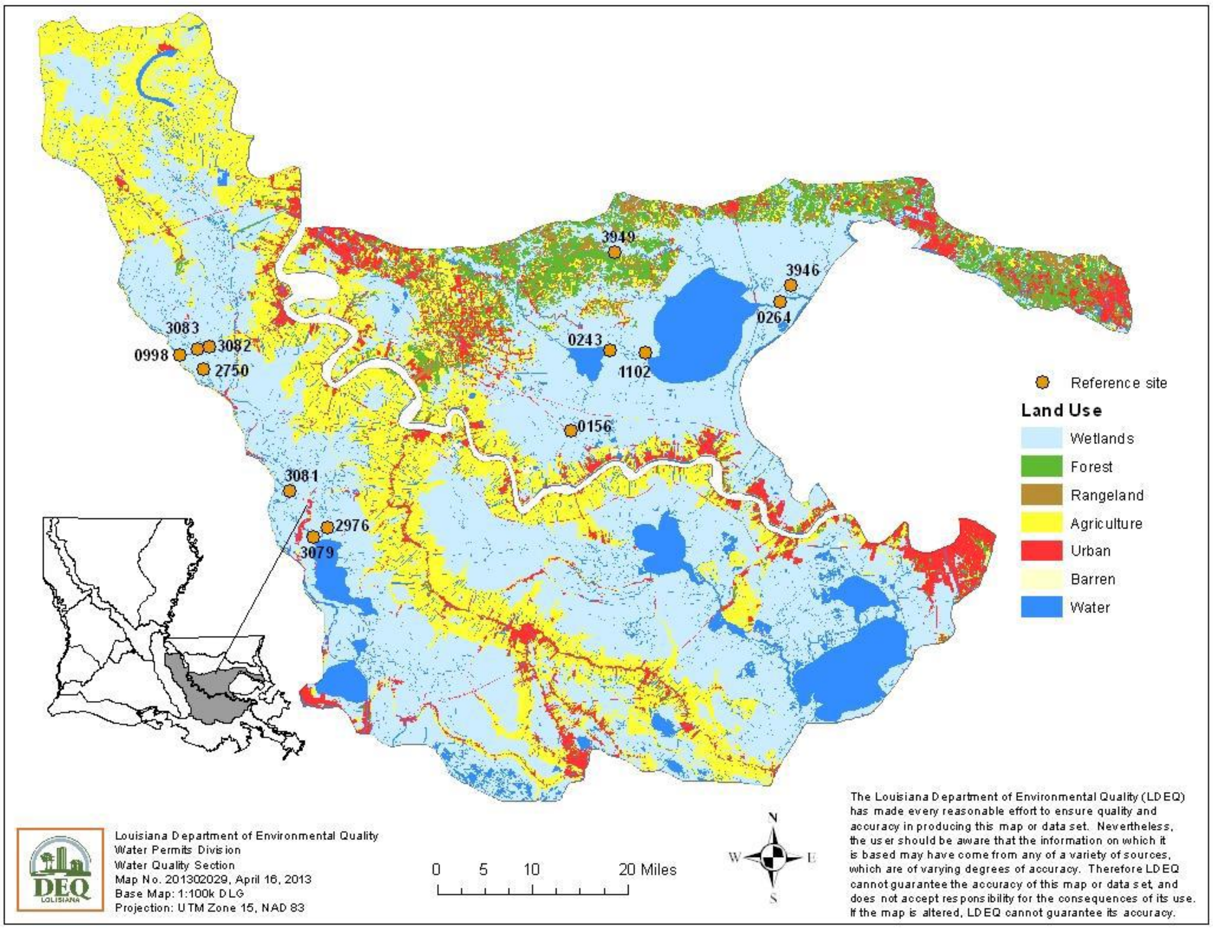


Table 2-1. LDEQ least-impacted sampling sites for the eastern LMRAPs ecoregion.

Section	LDEQ Site Number	Site Name	Subsegment	Water Body Type	UTM E
Eastern	3949	Tickfaw River	LA040502	STREAM	725188.02
	0243	Blind River east of Gonzales, LA	LA040403	STREAM	725017.19
	3946	Middle Bayou near Manchac, LA	LA040601	STREAM	754850.78
	0156	Blind River at Gramercy, LA	LA040403	STREAM	718531.00
	1102	Blind River near confluence with Lake Maurepas	LA040401	STREAM	731015.00
	0264	Pass Manchac at Manchac, LA	LA040601	STREAM	753911.26

2.4.1 Habitat Assessments

Habitat assessments provided a qualitative evaluation of physical conditions at reference sites. In the LMRAP Ecoregion, the predominant surrounding land uses were forests and wetlands. Local watershed erosion was considered to be slight and no hydromodifications were present at the reference sites. Reference streams in the LMRAP Ecoregion were rated as Fair to Excellent. Qualitative habitat assessments at reference sites indicated that these sites in the LMRAP ecoregion are of reference quality.

2.4.2 Chemical Data

The LDEQ collected continuous monitoring water quality data from May 2005 to February 2008 at eight stream sites in the western LMRAP as part of the BTUAA, from January to May 2010 at the same sites as well as eight sites in the eastern LMRAP, and again from March to December 2012 at six stream sites in both the eastern and western LMRAP. Water quality measurements included DO (mg/L), temperature (ºC), pH, specific conductivity (µS/cm), salinity (ppt), and percent DO (% saturation). Continuous monitors were deployed for 24 to 72 hours to collect diurnal data.

2.4.3 Physical Data

The LDEQ adapted the Low Gradient Stream Habitat Assessment form from USEPA's Rapid Bioassessment Protocols (Barbour et al. 1999) for use in the BTUAA (LDEQ 2007) as guidance for assessment of the habitat quality and stream characteristics of Louisiana's low gradient streams. These habitat assessments were carried out at the least-impacted stream sites identified for the LMRAP ecoregion from May 2005 to February 2008, January 2010 to May 2012, and March to December 2012.

In these assessments, key parameters (e.g., local watershed erosion and nonpoint source pollution; proportion of organic and inorganic streambed substrate; stream velocity; instream cover and substrate composition; channel morphology; and riparian and bank structure) were identified to provide a consistent assessment of habitat quality. Other qualitative measurements were estimated by LDEQ field staff for the following variables: predominant surrounding land use, canopy cover, hydromodifications, accessibility, recreational activities, water clarity and color, and percent composition of inorganic and organic substrate. This information was used to confirm that all least-impacted sites remained so and to make qualitative comparisons between the eastern and western subecoregions.

Habitat assessments were completed for all sites to verify or revoke the least-impacted site status of an area (i.e., ensure that all site selection criteria are still met during the sampling timeframe) as well as to make qualitative comparisons between the eastern and western subecoregions. Site information and survey conditions were documented during each sampling event using LDEQ's Site Information form (LDEQ 2009).

2.4.4 Biological Data

LDEQ fish sampling occurred between 2005 and 2006 in the western subecoregion as part of the BTUAA and during 2010 and 2012 in both subecoregions. A total of 10 least-impacted stream sites were sampled during this time period. Fish data were collected between the months of March and October, primarily using electroshocking and hoop nets with limited seining. Collection methods were consistent with protocols implemented in previous Louisiana ecoregion studies (DeWalt, 1995; DeWalt, 1997; LDEQ, 1996; LDEQ, 2009). Fish data were used to calculate species richness, total abundance, and species relative abundance.

Over 120 species of fish were observed from all reference waterbodies surveyed in the Barataria and Terrebonne Basins. The species richness (number of species observed) at each of the surveyed reference locations ranged from 8 to 64 species with a mean species richness of 21. Key species, or those typically observed at reference sites in the Coastal Deltaic and Lower Mississippi River Alluvial Plains Ecoregions in Louisiana, were present in all of the waterbodies examined.

The percent composition of freshwater, estuarine, and mixed freshwater/estuarine fish species for sites sampled by LDEQ was as expected, given characteristics of the salinity regimes and waterbody types examined in this study. That is, fish species compositions corroborate the site classifications based on observations of vegetation and salinity. Although fish population diversity in estuaries can vary due to seasonal migration and other seasonal or weather-driven changes in salinity, diversity indices are widely used for comparisons among locations and as indicators of impact due to pollution or hydrologic alterations (Thompson and Fitzhugh 1986; Davis and Simon 1995).

The Shannon-Wiener Diversity Index values calculated by LDEQ were found to be near 2 or above for most locations. Typical values are generally between 1.5 and 3.5 in most ecological studies, and the index is rarely greater than 4. The index increases as both the richness and the evenness of the community increase. Estimates of fish community composition indicate that fish species found at reference locations in the Barataria and Terrebonne Basins were representative of the fish community found at other LDEQ reference sites in the CDP and LMRAP Ecoregions. These results support the conclusion that the reference sites included in this survey are representative of least-impacted areas in the Barataria and Terrebonne Basins.

2.3 Data Analysis

2.3.1 Eastern and Western Subecoregion Comparisons

In 2012, LDEQ made a decision to limit the scope of the project to DO criteria refinements of streams in the eastern LMRAP with a focus on verifying similarities between eastern and western subecoregions. Together, the information described above was used to both qualitatively and quantitatively compare the eastern and western subecoregions of the LMRAP to determine if they are ecologically similar or dissimilar. LDEQ carried out a series of statistical tests to compare DO concentrations between the subecoregions, while more general comparisons were made for other water quality parameters as well as for habitat observations and fish community measurements. Prior to all statistical tests, data were truncated to increments of 24 hours.

LDEQ observed similarities between western and eastern portions of the LMRAP ecoregion in DO, pH, DO percent saturation, temperature, inorganic/organic content composition, fish species richness, and fish total abundance. There were no statistically significant dissimilarities observed between the western and eastern portions of the LMRAP.

2.3.2 Critical Period for DO

The critical period was determined through aggregation of reference stream continuous monitoring data by ecoregion and waterbody type as described in LDEQ (2008a). DO values were compared to the EPA's nationally recommended DO criteria for freshwater and marine waters (5 mg/L) as well as estuarine waters (4 mg/L). The critical period is defined as the month when data points for DO fall below the EPA-recommended criteria and ends when data points for DO no longer fall below the national benchmark (LDEQ 2008a). While DO was the primary data source LDEQ relied on for determination of critical period, biological information such as timing of fish spawning was considered during the critical period determination process.

In the BTUAA, DO in the western LMRAP fell below the EPA-recommended criteria of 5 and 4 mg/L during all months except February. Although DO did drop below the EPA recommendations in January and December, given the temperature observed in these months (less than 16 ºC) and potential timing of fish spawning (see LDEQ 2008a), these months were not considered to be part of the critical period. The critical period was determined to be March through November for streams in the western LMRAP ecoregion, while the non-critical period was determined to be December through February.

To identify the critical period for DO in the eastern subecoregion, continuous monitoring data collected in 2010 and 2012 were analyzed and compared to the national recommended criteria of 5 mg/L and 4 mg/L for freshwater, estuarine, and marine waters. DO in the eastern LMRAP fell below EPA's nationally recommended criteria of 5 and 4 mg/L throughout the year. Based on the scientific rationale used in the BTUAA, the critical period for streams in the eastern LMRAP ecoregion was also determined to be March through November; the non-critical period running from December through February.

2.4 Recommendation for Site-Specific DO Criteria

Extensive statewide DO monitoring has demonstrated that using EPA's nationally recommended criteria of 5.0 mg/L freshwater and 4.0 mg/L estuarine does not reflect naturally-occurring conditions that result in DO levels below the existing criteria for a substantial number of Louisiana waterbodies.

The results of the BTUAA and eastern LMRAP UAA outlined above indicate that EPA's nationally recommended DO criteria are not reflective of the naturally-occurring low DO conditions in waterbodies in the LMRAP Ecoregion. DO minimums were below the benchmarks during the critical periods for all waterbody types examined. The DO was especially low in the LMRAP Ecoregion, where minimum values were typically below 1 mg/L during the critical period. Based on both qualitative and quantitative comparisons of the eastern and western subecoregions of the LMRAP, no qualitative or statistically significant differences were observed between the two subecoregions. Therefore, the criteria established for streams in the BTUAA in the western subecoregion are considered appropriate for streams in the eastern subecoregion.

All continuous monitoring water quality data was analyzed by ecoregion and waterbody type to characterize the diurnal cycle present in some Louisiana waterbodies. A subset of the data, collected between 6 am and 12 pm, was analyzed separately. This time period was chosen because 1) low DO typically occurs in the morning hours from 12 am to 12 pm, and 2) ambient grab samples collected for assessment purposes are collected during the 6 am to 12 pm time frame. The data subset from 6 am to 12 pm represented water quality conditions to which grab samples were compared for assessment purposes to determine if the DO criterion would be met.

At those sites where DO minimum was below 1 mg/L, biological data indicate that fish are abundant. In addition, richness (number of species observed) ranged from 17 to 22 species in samples collected from LMRAP stream sites during periods when DO was below the national benchmark. The biological data collected supports that in these ecoregions diverse fish species are abundant in areas with low DO. Given that the fish and wildlife propagation use is supported in these reference areas of naturally low DO; it was reasonable for LDEQ to adopt site specific DO criteria specific to the LMRAP.

Table 1-2. Summary of the 10th percentile of the DO (mg/L) for waterbody types in the Lower Mississippi River Alluvial (LMRAP) Plains Ecoregion in the Barataria and Terrebonne Basins and comparison to EPA's nationally recommended criteria.

Ecoregion	Waterbody Type	Period	National Benchmark (mg/L)	10th percentile of reference data (6 am to 12 pm)	Criteria
LMRAP	Stream	Critical	5	2.3	2.3
LMRAP	Stream	Non-Critical	5	5.4	5.0

The 10th percentile of data collected in the eastern LMRAP between 6 am and 12 pm (per MOA and BTUAA protocols) is slightly lower than the proposed criteria revisions and therefore supports the use of the BTUAA criteria in the eastern LMRAP. The proposed criteria revisions are also supported by the findings of Justus et al. (2012) in which fish community changes were observed at a DO concentration of 2.3 mg/L. Based on this analysis, LDEQ proposed stream criteria for DO consistent with the values established in the BTUAA (2.3 mg/L; see LDEQ 2008a).

3.0 Action Area and Species Status

3.1 Description of Action Area

The action area is defined as "all areas to be affected directly or indirectly by the federal action and not merely the immediate area involved in the action" (50 CFR 402). The action is EPA's approval of Louisiana's site specific water quality criteria for DO for specific segments within the eastern LMRAP Ecoregion.

The LMRAP Ecoregion is a low-lying area of Louisiana that is bisected by the Mississippi River. The Barataria-Terrebonne watershed is in the western subecoregion, which was addressed in the BTUAA, the eastern LMRAP Ecoregion located to the east of the Atchafalaya River levee system and to the north of the Intracoastal Waterway. Many of the streams in this ecoregion have been hydrologically modified (LDEQ 1992). The portion located to the east of the Mississippi River (i.e., the eastern "subecoregion") is the action area.

3.2 Scope of Analysis

The EPA requested and received current ESA species lists for the eastern LMRAP action area from the USFWS and NMFS (Appendix B). Table 3-1 shows the combined list of the species identified by the USFWS and NMFS. Table 3-2 provides the Federal Register final rule notice for species with critical habitat that lie partially or fully within the action area.

Table 3-1. Species Listed Under the ESA within the Defined Action Area

Birds	**Status**	**Has Critical Habitat**	**Condition(s)**
Piping Plover *(Charadrius melodus)* Population: except Great Lakes watershed	Threatened	Final designated	
Red Knot *(Calidris canutus rufa)* Population: Wherever found	Threatened		
Red-Cockaded woodpecker *(Picoides borealis)* Population: Wherever found	Endangered		
Clams / Mussels			

Alabama (=inflated) heelsplitter *(Potamilus inflatus)* Population: Wherever found	Threatened		
Ferns and Allies			
Louisiana quillwort *(Isoetes louisianensis)* Population: Wherever found	Endangered		
Fishes			
Atlantic sturgeon (Gulf subspecies) *(Acipenser oxyrinchus) (=oxyrhynchus) desotoi)*	Threatened	Final designated	
Pallid sturgeon *(Scaphirhynchus albus)* Population: Wherever found	Endangered		
Mammals / Marine Mammals			
West Indian Manatee *(Trichechus manatus)* Population: Wherever found	Endangered	Final designated	
Fin whale (*Balenoptera physalus*)	Endangered		
Sei whale (*Balaenoptera borealis*)	Endangered		
Sperm whale (*Physter macrocephalus*)	Endangered		
Reptiles/Sea Turtles			
Gopher tortoise *(Gopherus polyphemus)* Population: West of Mobile and Tombigbee Rivers	Threatened		

Hawksbill sea turtle *(Eretmochelys imbricata)* Population: Wherever found	Endangered	Final designated	
Kemp's Ridley sea turtle *(Lepidochelys kempii)* Population: Wherever found	Endangered		
Leatherback sea turtle *(Dermochelys coriacea)* Population: Wherever found	Endangered	Final designated	
Loggerhead sea turtle *(Caretta caretta)* Population: Northwest Atlantic Ocean DPS	Threatened	Final designated	
Ringed Map turtle *(Graptemys oculifera)* Population: Wherever found	Threatened		
Green sea turtle *(Chelonia mydas)* Population: North Atlantic and South Atlantic Distinct Population Segments	Threatened		

Critical Habitat Designations

The following critical habitats lie fully or partially within the action area:

Table 3-2. Summary of Species Listed Under the ESA with Critical Habitat

Fishes			
Atlantic sturgeon (Gulf subspecies) *(Acipenser oxyrinchus) (=oxyrhynchus) desotoi)*	Threatened	Final designated	

3.3 Species Assessment

The EPA has made an assessment of all listed species included in the USFWS and NMFS species lists with ranges and/or critical habitat that overlap the action area to determine if exposure to minimum DO concentrations of 2.3 mg/L is Likely to Adversely Affect (LTAA), is Not Likely to Adversely Affect (NLTAA), or would have No Effect on listed species.

Only species that are dependent on water column DO for respiration are expected to potentially be directly affected by EPA's action. As a result, EPA has determined that its approval of the 2.3 mg/L DO criteria is NLTAA avian species, including the piping plover (*Charadrius melodus*), Red Knot (*Calidris canutus rufa*), and Red-Cockaded woodpecker (*Picoides borealis*). Given that the Louisiana quillwort (*Isoetes louisianensis*) is not dependent on DO concentrations, the 2.3 mg/L DO criteria is NLTAA the quillwort.

The West Indian manatee (*Trichechus manatus*) is found in fresh and estuarine waters within the eLMRAP. The manatee does not depend on water column DO for respiration and thus is NLTAA by the 2.3 mg/L DO criteria. The fin whale (*Balenoptera physalus*), Sei whale (*Balenoptera borealis*), and sperm whale (*Physter macrocephalous*) where identified by the NMFS as endangered in Louisiana. However, these cetacean species are not likely to be found in found in fresh and estuarine waters within the eLMRAP, but rather in Louisiana's coastal waters. Cetaceans are not dependent on water column DO for respiration, thus the 2.3 mg/L DO criteria is NLTAA these species.

The hawksbill sea turtle (*Eretmochelys imbricata*), Kemp's ridley sea turtle (*Lepidochelys kempii*), leatherback sea turtle (*Dermochelys coriacea*), loggerhead sea turtle (*Caretta caretta*), ringed map turtle (*Graptemys oculifera*), and green sea turtle (*Chelonia mydas*) were identified by both NMFS and the USFWS as potentially occurring within the action area. Although likely infrequent, these sea turtle species may enter Lake Pontchartrain and possibly the fresh and estuarine waters within the eLMRAP action area. The gopher tortoise (*Gopherus polyphemus*) is a dry-land tortoise with habitat found in the northeastern edge of the eLMRAP. Like the sea turtles, the gopher tortoise is not dependent on water column DO for respiration and thus the 2.3 mg/L DO criteria is NLTAA these species.

This biological evaluation will focus on the listed species that have the potential to be affected by fluctuations in DO levels within the water column. Listed species potentially affected by the proposed action include the Atlantic sturgeon (Gulf subspecies) (*Acipenser oxyrinchus*) (=*oxyrhynchus desotoi*), the Pallid sturgeon (*Scaphirhynchus albus*) and the Alabama (=inflated) heelsplitter (*Potamilus inflatus*).

The USFWS and NMFS share jurisdiction on Gulf sturgeon. As identified in Table 2, Gulf sturgeon critical habitat (50 CFR 226.214) occurs within the action area. The NMFS informed EPA (Bernhart 2017) that the USFWS is responsible for consultations regarding Gulf sturgeon and critical habitat in riverine units and estuarine units for EPA actions, the action area is limited to riverine and estuarine areas and therefore, based on the information we have received from NMFS, is under FWS jurisdiction for Gulf sturgeon.

4.0 Species Status and Life History

4.1 Gulf sturgeon (*Acipenser oxyrhynchus desotoi*)

Species Description

The Gulf sturgeon (*Acipenser oxyrhynchus desotoi*) is a subspecies of the Atlantic sturgeon (*Acipenser oxyrhynchus*). The Gulf sturgeon is a primitive fish characterized by bony plates, or scutes, a hard, extended snout and a heterocercal caudal fin - their tail is distinctly asymmetrical with the upper lobe longer than the lower. Adults range from 4-8 feet (1-2.5 m) in length;

females attain larger sizes than males. Gulf sturgeon are bottom feeders, and eat primarily macroinvertebrates, including brachiopods, mollusks, worms, and crustaceans. All foraging occurs in brackish or marine waters of the Gulf of Mexico and its estuaries; sturgeons do not forage in riverine habitat. Gulf sturgeons migrate into rivers to spawn in the spring; spawning occurs in areas of clean substrate comprised of rock and rubble. Their eggs are sticky, sink to the bottom, and adhere in clumps to snags, outcroppings, or other clean surfaces. They can live for up to 60 years, but average about 20-25 years.

Habitat

Historically, the Gulf sturgeon occurred from the Mississippi River to Tampa Bay, Florida. Today the species occurs throughout this range, but in greatly reduced numbers. The Gulf sturgeon is confined to the eastern Gulf of Mexico, possibly because this portion of the Gulf has predominately hard bottoms that are better suited to the Gulf sturgeon's feeding habits. The western Gulf has mostly mud, clay, and silt bottom sediments. (Barkuloo 1988).

Gulf sturgeons are anadromous, with adults spawning in freshwater and migrating to marine waters in the fall to forage and overwinter. Juvenile Gulf sturgeons stay in the river for about the first 2-3 years. Gulf sturgeons return to their natal stream to spawn. Riverine habitats where the healthiest populations of Gulf sturgeon are found include long, spring-fed, free-flowing rivers, typically with steep banks, a hard bottom, and an average water temperature of 60-72 °F. Gulf sturgeons initiate movement up to the rivers between February and April and migrate back out to the Gulf of Mexico between September and November.

The distribution of Gulf sturgeon within a given habitat is dependent on the physical characteristics of an area, including depth, substrate and water velocity. The characteristics of the preferred habitats of sturgeon and paddlefish for spawning are at a depth of 5.5- 8.1 meters and a temperature 18.3 °C (Smith and Clugston 1997). There is a preference for a depth of 8.4 meters and 7.5-15 °C, and limestone substrates in the adult life stage (Wooley and Crateau 1985, Smith and Clugston 1997, Fox et al. 2000).

Critical Habitat

In 2003, NMFS and the USFWS jointly designated **Gulf sturgeon critical habitat** in 14 geographic areas from Florida to Louisiana, encompassing spawning rivers and adjacent estuarine areas.

Distribution

The Gulf sturgeon is less widely distributed compared to the northern species. The Gulf sturgeon is restricted to the Gulf of Mexico in coastal waters from Tampa Bay, Florida, west to the mouth of the Mississippi River (Smith and Clugston 1997). The current range of the Gulf sturgeon appears to be from the Suwannee River, Florida, to eastern Louisiana (Wooley 1985).

Population Trends

The total number of adult Gulf sturgeon is unknown. However, over 15,000 adults are estimated in the seven coastal rivers of the Gulf of Mexico. Of those rivers, over 9,000 are estimated in the Suwannee River (GA-FL), the most viable subpopulation. About 3,000 mature Gulf sturgeons are estimated in the Choctawhatchee River (AL-FL). About 400 on average are estimated for each of the Pascaguola, Escambia, Yellow, Apalachicola and Pearl Rivers. The Pearl River is within the defined action area.

Threats
Historically, overfishing, throughout most of the 20th century has been significant. Current threats include construction of water control structures, such as dams and sills (mostly after 1950), exacerbated habitat loss, dredging, groundwater extraction, irrigation, flow alterations and poor water quality and contaminants, primarily from industrial sources.

Conservation Efforts
On September 30, 1991, the Gulf sturgeon was listed as a threatened species under the Endangered Species Act (ESA) (56 FR 49653). In 1995, a **Recovery and Management Plan** was published for the Gulf sturgeon. In addition, all U.S. fisheries for the Gulf sturgeon have been closed.

Regulatory Overview
The NMFS and USFWS share jurisdiction of this species. The 1995 joint **Recovery and Management Plan** was completed as noted previously. In 2003, C**ritical Habitat** for Gulf sturgeon was designated for 14 geographic areas among Gulf Of Mexico Rivers and tributaries.

4.2 Pallid sturgeon (*Scaphirhynchus albus*)

Species Description
The Pallid sturgeon (*Scaphirhynchus albus*), like other sturgeon species, is a primitive fish characterized by a flattened shovel-shaped snout, a long, slender, and completely armored caudal peduncle and absence of spiracle (Forbes and Richardson 1905). As with other sturgeon, the mouth is toothless, protrusible and ventrally positioned under the head. The skeletal structure is primarily cartilaginous.

The Pallid sturgeon inhabits areas of rapid current and prefers turbid water conditions (Kallemeyn 1983) that are necessary to conceal it from prey species (Mayden and Kuhajda 1997a). These conditions are historically found in the Missouri and lower Mississippi rivers (Mayden and Kuhajda 1997a). The Pallid sturgeon is associated with habitats characterized by sand, gravel or rocky substrates (Mayden and Kuhajda 1997a). They are well adapted to life on the bottom and inhabit areas of swifter water than does the related but smaller shovelnose sturgeon (Forbes and Richardson 1909; Carlson et al. 1985).

Data on food habits of age-0 Pallid sturgeon are limited. In a hatchery environment, exogenously feeding fry (fry that have absorbed their yolk and are actively feeding) will readily consume brine shrimp, suggesting zooplankton and/or small invertebrates are likely the food base for this age group. Juvenile and adult Pallid sturgeon diets are generally composed of fish and aquatic insect larvae with a trend toward piscivory as they increase in size (Carlson and Pflieger 1981; Hoover et al. 2007; Gerrity et al. 2006; Grohs et al. 2009; Wanner 2006; French 2013).

Pallid sturgeon can be long-lived, with females reaching sexual maturity later than males

(Keenlyne and Jenkins 1993). Based on wild fish, estimated age at first reproduction was 15 to 20 years for females and approximately 5 years for males (Keenlyne and Jenkins 1993). Like most fish species, water temperatures influence growth and maturity.

Females do not spawn each year (Kallemeyn 1983) and fecundity is related to body size. Spawning appears to occur between March and July, with lower latitude fish spawning earlier than those in the northern portion of the range. Adult Pallid sturgeon can move long distances upstream prior to spawning; a behavior that can be associated with spawning migrations (U.S. Geological Survey 2007; DeLonay et al. 2009). Spawning appears to occur adjacent to or over coarse substrate (boulder, cobble, gravel) or bedrock, in deeper water, with relatively fast, converging flows, and is driven by several environmental stimuli including day length, water temperature, and flow (U.S. Geological Survey 2007; DeLonay et al. 2009).

Habitat
Pallid sturgeons are bottom-oriented, large river obligate fish inhabiting the Missouri and Mississippi rivers and some tributaries from Montana to Louisiana (Kallemeyn 1983). Pallid sturgeon primarily utilizes main channel, secondary channel, and channel border habitats. Throughout their range, juvenile and adult Pallid sturgeons are rarely observed in habitats lacking flowing water which are removed from the main channel (i.e., backwaters and sloughs). Specific patterns of habitat use and the range of habitat parameters used may vary with availability and by life stage, size, age, and geographic location.

Much of the habitat usage data for Pallid sturgeon are based on habitat characterizations in altered environments, in some cases substantially altered environments, including an altered hydrograph and temperatures, suppression of fluvial processes, stabilized river banks, loss of natural meanders and side channels, fragmented habitats, and increased water velocities. In the portions of the lower Mississippi, Pallid sturgeons have primarily been captured near engineered channel, steep sloping banks, and channel border areas (Killgore et al. 2007b; Schramm and Mirick 2009).

Pallid sturgeons are primarily benthic fish, and have been documented in waters of varying depths and velocities. This species is typically found in areas with relative depths at 75% of the cross section (Constant et al. 1997; Gerrity 2005; Jordan et al. 2006; Wanner et al. 2007). Bottom water velocities associated with collection locations are generally < 1.5 m/s (4.9 ft/s) with reported averages ranging from 0.58 m/s to 0.88 m/s (1.9 ft/s to 2.9 ft/s) (Carlson and Pflieger 1981; Elliott et al. 2004; Erickson 1992; Jordan et al. 2006; Swigle 2003; Snook et al. 2002).

Critical Habitat
No critical habitat has been designated.

Distribution
Since listing in 1990, wild Pallid sturgeon historical range extends from the headwaters of the Missouri River from in Montana, downstream through North Dakota, South Dakota, Nebraska and Missouri. The historical range includes the Mississippi River from the Iowa-Missouri line through Missouri, bordering Illinois, Kentucky, Arkansas, Mississippi through Louisiana. Pallid

sturgeon observations and records have increased with sampling effort in the Mississippi River basin. In 1991, the species was identified in the Atchafalaya River, Louisiana (Reed and Ewing 1993). Additionally, the species has been documented in the Red River, Louisiana (Slack et al. 2012)

Population Trends

As many as 2,750 to 4,100 Pallid sturgeons remain in the Atchafalaya River in Louisiana. However, for the greatest part of the contiguous range, the lower Missouri River below Gavins Point Dam, downstream to the Mississippi River and downstream to the Gulf of Mexico, no estimates are available. The best estimate, at present, of the total population of Pallid sturgeon is that as few as 6,000 to as many as 21,000 may still exist throughout the entire range of this species (Duffy *et al.* 1996).

There are indications that the northern and southern Pallid sturgeon arose independently from different ancestors and are not a monophyletic lineage, thereby representing two separate species (Campton et al. 2000). These data (Campton et al. 2000, Tranah et al. 2001, Heist and Schrey 2006a) suggest that the genetic structuring within the Pallid sturgeon's range represents two distinct groups at the extremes of the species range with a middle intermediate group representing the lower Missouri and middle Mississippi Rivers. This pattern is suggestive of a pattern of isolation by distance, with gene flow more likely to occur between adjacent groups than among geographically distant groups resulting in greater genetic differences as geographical distance between groups increases.

Threats

There are known and potential threats that affect the habitat or range of Pallid sturgeon. Habitat modification, including the construction of dams on the Missouri River and extensive channelization in the lower Missouri and Mississippi Rivers, is the primary factor affecting the continued existence of this species. Related altered and/or degraded water quality and altered flows are considered detrimental. Pollution may be a serious threat over a portion of its range. Past commercial harvest may have surpassed replenishment capability and commercial harvest may still pose a threat. This species is known to hybridize with the closely related and more abundant shovelnose sturgeon (*S. platorynchus*) where their ranges overlap.

Conservation Efforts

The Pallid sturgeon (*Scaphirhynchus albus*), was listed as an endangered species on September 6, 1990 (55 FR 36641). The Pallid sturgeon is found in the Missouri and Mississippi River basins. The original Pallid sturgeon recovery plan was approved in 1993. The revised recovery plan was published on March 4, 2014 (79 FR 12213) and documents the current understanding of the species life history requirements, identifies threats to the species, includes revised recovery criteria, and describes those actions believed necessary to eventually delist the species.

The primary strategy for recovery of Pallid sturgeon is to: 1) conserve the range of genetic and morphological diversity of the species across its historical range; 2) fully quantify population demographics and status within each management unit; 3) improve population size and viability within each management unit; 4) reduce threats having the greatest impact on the species within each management unit; and, 5) use artificial propagation to prevent local extirpation within management units where recruitment failure is occurring.

Regulatory Overview

A **Revised Recovery and Management Plan** was published on March 4, 2014 (79 FR 12213). The USFWS has jurisdiction over this species. The USFWS completed a **5-year Review** of Pallid sturgeon in January 2014.

4.3 Alabama (=*inflated) heelsplitter (Potamilus inflatus*)

Species Description

The Alabama (=inflated) heelsplitter (*Potamilius inflatus*) is a freshwater mussel which reaches a maximum adult shell size of about 140 millimeters (mm) or 5 ½ inches (in) in length. The heelsplitter is clearly distinguishable by shell morphology. The shell is brown to black and may have green rays in young individuals. The heelsplitter has an oval, compressed to moderately inflated, thin shell. The valves may gape anteriorly, the umbos are low, and there is a prominent posterior wing that may extend anterior to the beaks in young individuals. The umbonal cavity is very shallow, and the nacre is pink to purple. Maximum shell length is about 140 millimeters (5 1/2 inches) in adults (Stern 1976). It is most similar to the pink papershell (*Potamilus ohioensis*), yet is easily distinguished by shell morphology (Hartfield 1988). The heelsplitter appears more inflated due to a more developed and rounded posterior ridge. The posterior wing of the heelsplitter is more pronounced and abruptly rounded over the dorsum.

The life history of this species is largely unknown. Gravid females have been collected from the Amite River, Louisiana, during October (Hartfield 1988). At that time, they were observed to extend a mantle margin just above the substratum surface in shallow, clear water. This behavior is similar to some species of *Lampsilis* and has not been reported for any species of *Potamilus*. With the exception of these few observations, the life history is presumed to be similar to that of other unionids. During the spawning period, males discharge sperm into the water and females collect the sperm by the siphoning process. Eggs are fertilized and held in the female’s gills where they develop into larvae or glochidia. The glochidia are discharged into the water where they attach to a fish host, become encysted, and metamorphose into juvenile mussels that are capable of surviving if they fall to suitable substrata. Mussels are also dependent upon the water currents to bring food particles within the range of their siphons. Investigations by Roe et al. (1997) found the freshwater drum (*Aplodinotus grunniens*) to be a suitable fish host for the glochidia of this species.

Habitat

The preferred habitat of this species is soft, stable substrata in slow to moderate currents (Stern 1976). It has been found in sand, mud, silt and sandy-gravel, but not in large or armored gravel (Hartfield 1988). It is usually collected on the protected side of bars and may occur in depths over 6 meters (20 feet). The occurrence of this species in silt does not necessarily indicate that the life cycle can be successful in that substratum (Hartfield 1988). Adult mussels may survive limited amounts of silt, whereas juveniles would suffocate. The occurrence of this species in silt may be because it was established prior to deposition of the silt.

Critical Habitat
None delineated.

Distribution
The Alabama heelsplitter historically occurred in the Tangipahoa and Pearl Rivers in southeastern Louisiana. The presently known distribution is limited to the Amite River, Louisiana, and the Tombigbee and Black Warrior Rivers, Alabama (Stern 1976, Hartfield 1988). The collection of this species from the Pearl River by Hinckley was reported by Frierson (1911) and a single example collected by Parker is housed in the U.S. National Museum of Natural History (Dr. James Williams, U.S. Fish and Wildlife Service, pers. comm. 1988). There are no other reported collections from the Pearl River (Hartfield 1988). A single live specimen was collected from the Tangipahoa River, Louisiana, in 1964 by Stein and Stansbery (Dr. David Stansbery, Ohio State University, pers. comm. 1985). Hartfield (1988) did not find the species in the Tangipahoa River during his survey. Hurd (1974) doubted the occurrence of this species in the Coosa River based upon the single lot available in museums. Reports of *Potamilus alatus* from the Coosa River may actually be *P. inflatus*. However, neither species has been reported from the Coosa or Alabama Rivers for an extended period (Hurd 1914, Hartfield 1988).

In the Amite River, the heelsplitter occurs in the lower and mid reaches between State Highways 10 and 42 (Hartfield 1988). In the Tombigbee River, the heelsplitter occurs in Gainesville Bendway; downstream of Coffeeville and Demopolis Dams; and in the vicinity of the Naheola Bridge (River Mile 173). It is likely the heelsplitter occurs in any suitable habitat between Demopolis Dam and the downstream impoundment effects of Coffeeville Dam. In the Black Warrior River, this species is known to occur from Demopolis Lock 5 upstream to Selden Dam, near Eutaw, Alabama. Two individuals were recently discovered at River Mile 300.5 in Tuscaloosa County, Alabama. The increased range in the Tombigbee and lower Black Warrior Rivers is an expansion over that known when the heelsplitter was listed and is the result of intensive surveys by Service biologists. The recent collection from the Black Warrior River, Tuscaloosa County, was by Dr. John C. Hall, Alabama Museum of Natural History (Stuart McGregor, Geological Survey of Alabama, in litt. 1992). The extent of this most recently discovered population is unknown.

Population Trends
Population numbers are low, however, the species has more than 50 miles of available habitat. Exact population numbers are unavailable. The U. S. Army Corps of Engineers found 63 live animals during their surveys of the Tombigbee and Black Warrior Rivers (Miller, 1995). In the Black Warrior-Tombigbee waterway densities ranged from 0.00 to 1.73 per 100 square meters. Extensive surveys of the Alabama River have located only a single fresh dead shell. Extensive surveys of the Pearl River have resulted in the collection of only a few fresh dead shells (USFWS, 2000). Viability in Louisiana is not known but it is believed to be extirpated or nearly so (Jones et al., 2005).

Recent surveys have found the species to be doing better than previously believed in the Black Warrior River (Paul Hartfield - pers. comm., 1994). The total range has been decreased, however, with the Amite River populations questionable and the Alabama River population likely not viable. Mississippi populations are likely extirpated (Jones et al., 2005) as are occurrences in the Alabama River drainage in Alabama (Mirarchi et al., 2004).

Threats

The Alabama heelsplitter was listed because of habitat degradation that has resulted in the restriction of this species to limited stretches of three river systems and also because of the continued threats to these populations. In the Amite River, there is a continued and serious threat from gravel mining that is largely unregulated. The populations in the mainstem of the Tombigbee River are affected to a limited extent by channel maintenance activities. In addition, the population below Coffeeville Lock and Dam is not very abundant. The population in Gainesville Bendway may be adversely affected by the regulation of water flows from Gainesville Dam. This structure is designed to allow the passage of normal river flows with the exception of water needed for lockage. During low flows, there is little, if any, water released over Gainesville Dam spillway for varying periods of time. This could result in very low DO conditions on the river bottom in Gainesville Bendway and adversely impact the heelsplitter.

The heelsplitter is threatened by sand and gravel mining in the Amite River and to a limited extent by channel maintenance in the Tombigbee and Black Warrior Rivers. Channel maintenance is a threat in the Tombigbee and Black Warrior Rivers, to the degree that mussel beds are suffocated with dredge disposal (USFWS, 1992). Occasional take by dredge in the Tombigbee and Black Warrior rivers, is probably of little consequence to the entire population of the species (USFWS, 1992).

In the Mobile River basin, the greatest threats are dams (for navigation, water supply, electricity, recreation, and flood control), channelization (causing accelerated erosion, altered depth; and loss of habitat diversity, substrate stability, and riparian canopy), dredging (for navigation or gravel mining), mining (for coal, sand, gravel, or gold) in locally concentrated areas, pollution-point source (industrial waste effluent, sewage treatment plants, carpet and fabric mills, paper mills and refineries in mainstem rivers), and nonpoint source pollution (construction, agriculture, silviculture, urbanization).

Conservation Efforts

Since its listing, Service biologists have extended the known range of the heelsplitter in the Black Warrior and Tombigbee Rivers. Collections by Service biologists have been in deep water, sometimes of 30 feet or more. Divers found this species in the Black Warrior River in the vicinity of Demopolis Lock 5 boat ramp (river mile 232-234.5) in deep water, and the heelsplitter likely occurs in suitable substrata throughout the entire 25 miles of the Black Warrior River downstream of Selden Dam. Service biologists also found the heelsplitter downstream of Demopolis Lock and Dam and in the vicinity of Naheola Bridge (River Mile 173) on the Tombigbee River suggesting that the species likely occurs in suitable habitat throughout the stretch between Demopolis Dam and the impoundment effects of Coffeeville Dam. Dr. John Hall collected two live specimens of the heelsplitter from the Black Warrior River (River Mile 300.5), Tuscaloosa County, in 1992. Both specimens were photographed and returned to the river. The heelsplitter is likely to be even more widespread in the mainstem Tombigbee and Black Warrior Rivers.

Service biologists have met with the New Orleans District Corps of Engineers to discuss gravel mining as a primary threat to this species in the Amite River in an effort to alleviate that threat through regulation, and they are working with the Mobile District Corps of Engineers to provide protection for this species in the Tombigbee and Black Warrior Rivers. The discovery of additional populations of the heelsplitter extends the protection of Sections 7 and 9 of the Endangered Species Act to those populations.

Regulatory Overview
The U.S. Fish and Wildlife Service (Service) (1990) determined the heelsplitter to be a threatened species on September 28, 1990.

5.0 Analysis of Effects

Natural conditions in coastal southeastern Louisiana in general, including the action area that is within the eastern LMRAP, are typified by black water bayous that often have little flow to tidally influenced backflow. Dense vegetation is a significant source of shade and organic material. Dissolved oxygen concentrations in these lowland streams are naturally lower than in upland streams as a consequence of substantial decomposition of this organic material and low aeration and flushing rates, particularly during the summer months. Native biota often have respiratory or physical adaptations that enable them to cope with these inherently harsh conditions and low DO concentrations (Eriksen et al., 1996; Val et al.., 1998).

This analysis is intended to determine the effect that the revised minimum DO criterion of 2.3 mg/L as applied between the months of March to November may have on the Atlantic sturgeon (Gulf subspecies) (*Acipenser oxyrinchus*) (=*Oxyrhynchus desotoi*) and the Pallid sturgeon (*Scaphirhynchus albus*) and the Alabama (=inflated) heelsplitter (*Potamilus inflatus*). The analysis of effects in the biological evaluation assumes that the species of interest are exposed to waters meeting the minimum DO criterion during the time frame specified and examines the likely effects on the species under that scenario.

5.1 Response to DO in the Gulf and Pallid Sturgeon

5.1.1 Distribution, Habitat and Movement

Although historical populations have been drastically reduced, sturgeons still maintain a wide distribution across North America. The individual distribution of sturgeons is directly related to the migratory strategies and habitats they have adapted to. A variety of habitats are essential for the different life stages of sturgeon, and though sturgeons spawn exclusively in freshwater, they can be found in fresh, brackish, and marine environments. Additionally, the migratory behaviors of sturgeon are complex and species specific. (Wilson and McKinley 2004).

The historical distribution and abundance of sturgeon and paddlefish across North America has been significantly reduced over time, primarily due to anthropogenic influences that have blocked migratory routes and destroyed essential habitats. (Wilson and McKinley 2004). All sturgeon and paddlefish migrate to avoid adverse conditions, ensuring successful reproduction and to optimize feeding (Auer 1996a, Bemis and Kynard 1997). The Pallid sturgeon remains in fresh water throughout their lives, while adult Gulf sturgeons begin upstream movements from the Gulf into coastal rivers in February when temperatures increase to 16 - 19 ^{o}C (Smith and Clugston 1997). As summer temperatures drop below 20 ^{o}C in November, Gulf sturgeons begin downstream migrations back to the Gulf of Mexico (Smith and Clugston 1997). As noted in the species description, Gulf sturgeons are restricted to the Gulf of Mexico in coastal waters from Tampa Bay, Florida, west to the mouth of the Mississippi River (Smith and Clugston 1997), and the current range of the Gulf sturgeon appears to be from the Suwannee River, Florida, to eastern Louisiana (Wooley 1985).

5.1.2 Environmental Requirements, Preferences, and Tolerance Limits

The action area defined as the eastern LMRAP ecoregion is characterized as a low-lying area with little or no flow or even backflow in tidally influenced segments with soft stream bottoms and naturally low DO concentrations. In order to understand the DO requirements for both the Gulf and Pallid sturgeon, it is necessary to understand their general environmental requirements and tolerance to differing stressors. Gulf and Pallid sturgeons occupy a variety of habitats with broad variations in exposure to light, temperature, DO and carbon dioxide concentrations, salinities, depths, and velocity conditions that are typical of eutrophic, nutrient-rich aquatic systems where a high biomass tends to overwhelm oxygen inputs from inflowing water, photosynthesis, and atmospheric diffusion.

5.1.2.1 Light

Photoperiodicity appears to regulate sturgeon growth and reproduction in a manner similar to that shown in salmonids. Cech (from LeBreton 2004) noted that light intensity and day length (photoperiod) influence behavior, growth, and reproduction of sturgeon as seen in other fish. Cultured white sturgeon swim undisturbed during portions of the day with low light intensity, but move to shaded tank areas when exposed to sunlight. Lankford et al. (2003) reported a more pronounced stress response (high plasma cortisol and lactate levels) in green sturgeon stressed at night, when compared with those stressed during the daytime.

5.1.2.2 Temperature

Environmental temperature controls metabolism, growth and reproduction in ectothermic fish (Brett 1979). Activity and growth of young sturgeons generally increase with temperature increases until an optimal temperature is reached, usually below 25 ^{o}C. The distribution range of North American sturgeons extends over a zone with the temperature variation up to 30 ^{o}C, but they generally prefer cool (e.g., <25 ^{o}C) temperature conditions. Gulf sturgeon adults and large juveniles move upriver from the Gulf in the spring when the water temperature is 15 to 20 ^{o}C (Chapman and Carr 1995, Sulak and Clugston 1998, Fox et al. 2000) and return to the Gulf in the fall when water temperatures range from 18 to 23 ^{o}C. Young life stages may be the most temperature-sensitive within sturgeon species. Laboratory studies show that Gulf sturgeon eggs, embryos, and larvae have the highest survival rates in the 15 to 20 ^{o}C range, and that survival decreased significantly at temperatures >25 ^{o}C (Chapman and Carr 1995).

Telemetry observations in Florida's Apalachicola River indicated late spring and summer habitat preferences include areas with sand and gravel substrate~~,~~ at an average depth of 8.4 m with an average water velocity of 0.64 $m \cdot s^{-1}$ (Wooley and Crateau 1985). A fall staging area in the Brothers River was characterized by substrates of sand and clay at depths of 11 m in velocities of 0.62 $m \cdot s^{-1}$ (Wooley and Crateau 1985). Fish overwintered in high velocity areas over 14 m deep in water temperatures of 7.5 - 15 ^{o}C (Smith and Clugston 1997). In the Suwannee River, Gulf sturgeon spawned in the upper reaches of the river when temperatures reached 18.3 ^{o}C (Smith and Clugston 1997). Fish in the upper Apalachicola River are speculated to spawn at water temperatures of 22.5 - 23 ^{o}C (Wooley and Crateau 1985), and the results of laboratory studies on early life stages agree with field observations on spawning temperature ranges of several stocks (Kohlhorst 1976, McCabe and Tracy 1994, Bruch and Binkowski 2002, Perrin et al. 2003).

Given that high temperatures are known to amplify negative effects of hypoxia on growth and survival of estuarine fishes (Coutant, 1985), Secor et al. (1998) investigated the effects of DO and temperature on growth, survival, and respiration of juvenile young-of-the-year (YOY) Atlantic sturgeon and hypothesized that they may be more susceptible than other estuarine fishes to high temperature and low oxygen conditions, now prevalent in the Chesapeake Bay. Experiments were conducted using four combinations of surface access, temperature, and DO, each replicated twice. To increase confidence in associating survival rates, treatments with surface access and high temperature (at both low and high DO levels) were repeated for a total of four replicates. (Secor et al. 1998).

Secor et al. (1998) reported that deaths were observed only in hypoxic conditions over a 42-hour treatment period. At hypoxic levels, survival was lower at 26 °C (mean=6.3% survival) than at 19 °C (mean =78.3% survival). In the 26 °C sealed-hypoxic level tanks (no air gap), all individuals died within the first 30 hours of the experiment under hypoxic conditions. In the unsealed tanks, dying sturgeon were observed at the air-water interface in unsealed tanks, or just below the lid in sealed tanks, indicating that temperature can exacerbate the effect of sustained hypoxic conditions (42-h). Secor et al. (1998) also reported that despite reduced survival and respiration in conditions of low DO, Atlantic sturgeons were able to reduce activity but still feed and allocate some energy to growth as seen in other sturgeon species. Cech et al. (1984) also observed continued growth by juvenile white sturgeon *(Acipenser transmontanus)* under hypoxic conditions.

The Secor et al. (1998) study suggests that juvenile Atlantic sturgeons are vulnerable to high temperature, hypoxic conditions, and while these conditions do occur naturally, it is important to keep in mind that controlled studies cannot reproduce natural conditions. The sealed tanks did not allow the subject fish to use any avoidance behaviors commonly seen in fish. Although the sturgeons in the unsealed tanks were reported to have moved to the surface interface, they could not move to an area with lower temperature or higher DO concentrations.

5.1.2.3 Salinity

Jenkins (1995) carried out a series of experiments to examine tolerance levels to increased salinity and low oxygen concentrations with cultured juvenile shortnose sturgeon (*A. brevirostrum*) in estuarine and near shore environments.

Jenkins reported that all age classes could tolerate salinities to 7 parts per thousand (ppt), but that the fish begin to decline at 9 ppt. Fish in both the 15 and 20 ppt treatment levels appeared to be behaving normally but each group had lost weight during the test. Jenkins also noted that 330-day-old fish were able to tolerate salinities up to 25 ppt for 18 hours but surmised that they could not tolerate salinities >30 ppt with the short acclimation they received in their tests. These data support the importance of estuarine habitat as nursery areas for juvenile shortnose sturgeon.

Jenkins et al. (1995) also carried out hypoxia tests that indicated that older shortnose sturgeon were better able to tolerate low oxygen levels than younger fish for short periods. In each test at a DO concentration of <3.0 mg/L, changes in behavior were noted. Jenkins noted that the fish would become immobile and the only movement that could be detected would be the rhythmic movements of the operculum as it pumped water over the fishes' gills. This behavior has been noted and examined in detail by researchers studying white sturgeon, *Acipenser transmontanus,* and appears to be an adaption to living and feeding on the bottom (Burggren and Randall 1978). Older and larger fish may be able to more efficiently pump water during hypoxic conditions, and this may be why older fish tolerated low DO concentrations better than the young fish in these experiments, and the response of decreasing to ceasing movement may be both a behavioral and metabolic response. It is important to note that these results are based on laboratory tests in static environments where DO concentrations are held at low levels for extended periods, and not necessarily indicative of natural conditions.

Niklitschek et al. (2009) carried out experiments focused on multiple ecophysiological responses to hypoxia, salinity and temperature. Although interactive effects of these three variables were suggested for most responses, the interactions were only significant for growth and routine metabolism. Comparing the deviance explained by each of these three environmental factors across measured responses, Niklitschek et al. observed that temperature was the most important explanatory factor for most responses, except specific dynamic action and egestion, where DO saturation (DO_{SAT}) was the most influential factor. DO and salinity explained similar proportions (24–30%) of observed variability in food consumption and growth responses, but the effects of salinity on routine metabolism were rather limited, explaining <10% of model deviance. The much larger effects of salinity on food consumption and growth indicate that salinity effects on fish bioenergetics can exceed what would be expected due to osmoregulation costs alone (Boeuf and Payan, 2001).

Tolerances to sub-lethal hypoxia were expected to increase as mass-specific oxygen demand by metabolism decreased with size (Ishibashi et al., 2005). Niklitschek observed an unexpected lack of an age-class effect on food consumption and growth responses to hypoxia, but this effect could be mitigated as oxygen delivery rates also decrease with size (Pauly, 1981). This suggested that the observed higher tolerance of larger organisms to hypoxia in the wild might be related to the ability to escape and/or to endure unfavorable conditions for longer periods (Breitburg, 1992), rather than to a higher physiological tolerance.

The additive and interactive effects found for DO, temperature and salinity could have major consequences for juvenile Atlantic sturgeon in the wild. In historical nursery areas, such as the Chesapeake Bay and other U.S. southeastern estuaries, high temperatures coincide with hypoxia every summer (Collins et al.., 2000; Niklitschek and Secor, 2005). Under this scenario the limiting effects of hypoxia would reduce physiological scopes to a point where the relative importance of salinity effects becomes critical. For instance, in Virginia estuaries that support juvenile Atlantic sturgeon, summer temperatures become optimal in freshwater, but brackish bottom waters are often hypoxic (<40% DO_{sat}). A potential refuge from sub-lethal conditions of high temperature and hypoxia exists in the lower Chesapeake Bay (due to marine influence), which is normoxic and cooler, but here salinity is optimal (Niklitschek and Secor, 2005). Hence a three-way "habitat squeeze" (Coutant, 1987) is possible, which could be further reduced by anthropogenic perturbations such as pollution, freshwater flow reductions and climate change (Niklitschek and Secor, 2005).

Niklitschek et al. suggest that it is important to consider temperature and salinity as relevant covariates for hypoxia criteria definitions, considering the effects of both on physiological rates and oxygen solubility in water and blood (Holeton and Randall, 1967). Niklitschek noted that if optimal growth or survival rates were used as criteria to set a hypoxia threshold for juvenile Atlantic sturgeon, that value would rise from 40 to 70% DO_{SAT} if temperature increased from 12 to 20 °C (Niklitschek et al. 2009). At a salinity of 1 ppt, these values would correspond to concentrations of 4.3 and 6.3 mg/L, respectively, while at a salinity of 29 ppt, the same thresholds would correspond to concentrations of 3.6 and 5.4 mg/L respectively. This Percent DO saturation or partial pressures of DO are the biologically relevant factors for hypoxia, since these, rather than oxygen concentration physically determine oxygen uptake from the surrounding water by fish (Cech, 1990; Kiceniuk and Colbourne, 1997).

Niklitschek et al.'s overall findings suggest that routine oxygen consumption was significantly affected by DO, temperature, salinity and fish mass. Further, a significant shift in growth responses with age indicated higher tolerance to salinity in yearlings than in YOY in Atlantic sturgeons. No other size-dependent changes were significant, either for hypoxia or for temperature effects. Survival tended to increase with DO saturation, and decreased at the highest temperature and salinity levels. These results indicate both additive and synergistic effects from temperature, salinity and DO as factors in ecophysiological responses.

5.1.2.4 Dissolved Oxygen

Crocker and Cech (1997) investigated routine oxygen consumption rates and swimming activity rates of juvenile white sturgeon using closed respirometers at what are considered life-interval-appropriate temperatures. The results were expressed as milligram (mg) oxygen consumed per gram body weight per hour "small" (0.2 g body weight at 10 ^{o}C), "medium" (1.9 g at 16 ^{o}C), and "large" (63 g at 20 ^{o}C), under normoxic (8.1-10 mg/L) and moderately hypoxic (4.6-5.7 mg/L) water conditions. The study reported that all juvenile white sturgeon displayed significant oxygen consumption rate decreases typical for each life stage. At this level of moderate hypoxia, activity significantly decreased at these temperatures and at 25 °C, at least partially explaining their decreased oxygen consumption rates (Crocker and Cech 1997). In turn, the decreased activity level may account for decreased food consumption rates and/or decreased energy storage (although these were not quantified), and thus the significantly slower growth in juvenile white sturgeon found to occur under mild hypoxia (58% of air-saturated conditions) in comparison to the growth rate under normoxic conditions at 15, 20, and 25 °C, (Cech et al. 1984). This hypometabolic response, seen also in adults (Burggren and Randall 1978), may benefit white sturgeon and other species like the Gulf and Pallid sturgeons in natural habitats where decreased activity would decrease oxygen demand, thereby conserving oxygen resources in hypoxic habitats until conditions improved.

Egg and larval development have also been reported as vulnerable to various forms of pollution and other water quality parameters (e.g., temperature, DO). Sulak et al. (2004) suggested that successful egg fertilization for Gulf sturgeon may require a relatively narrow range of pH and calcium ion concentration, but this response varies among sturgeon species. Juvenile white sturgeons display significant decreases in O_2 consumption rates with exposure to mild hypoxia representing 51% of air-saturation levels at temperatures typical for this life stage (Crocker and Cech 1997). Both juvenile and adult White sturgeon displayed a decrease in activity, food consumption and growth rates under mild hypoxia in comparison to the growth rate under normoxic conditions (58% of air-saturated conditions) at 15, 20, and 25 °C, (Cech et al. 1984).

The activity of Atlantic sturgeon (*Acipenser oxyrinchus*) and shortnose sturgeon (*A. brevirostrum*) did not change with exposure to moderate hypoxia (3 mg/L) at 15 ^{o}C (Daniel Baker, Dept. Biology, Univ. New Brunswick, Saint John, N.B. Canada; pers. comm.), with gill ventilator frequencies increasing by 50% in both species during hypoxic exposure. Plasma lactate concentrations, increased in both species after exposure to 2 mg/L at 15 ^{o}C, which is close to the minimum DO concentration (1 mg/L) measured in the Saint John River, indicating a partial shift to anaerobic metabolism (Daniel Baker, Dept. Biology, Univ. New Brunswick, Saint John, N.B. Canada; pers. comm.), suggesting that a similar hypometabolic response is probable in Gulf and Pallid sturgeons.

5.1.3 Swimming and Respiration

There are two types of physiological and metabolic responses to ambient oxygen levels possible in sturgeons. In one response, oxygen regulators adjust their ventilation rates to compensate for changing oxygen levels, which allows them to maintain a constant respiration rate and aerobic metabolism. This mechanism has likely evolved for maintaining oxygen uptake in situations such as bottom feeding where oxygen availability is compromised. Oxygen regulators can alternate between buccal and opercular water intake while feeding, to maintain oxygen uptake (Burggren and Randall 1978). The second response type is that of an oxygen conformer; those that allow a decline in their respiration rates as environmental oxygen decreases, with concomitant reductions in metabolic rate and aerobic metabolism. Most species of sturgeon are oxygen conformers although the range of oxygen concentrations varies with species and population (Burggren and Randall 1978; Ruer et al. 1987; Secor and Gunderson 1997). In oxygen conformers, aerobic metabolism may be replaced by anaerobic mechanisms. Reduced metabolism at low oxygen concentrations has been attributed to reduced activity in white sturgeons (*Acipenser transmontanus;* Crocker and Cech 1997).

Several studies have examined the relationship between activity, respiration and ambient oxygen levels for sturgeon. Burggren and Randall (1978) found that, under normoxic conditions, white sturgeon respiration was very similar to that displayed by teleosts (where mean branchial water flow was measured at 350 mL/kg/min, oxygen utilization, or the relative amount of oxygen taken up by the gills, was 30 to 40%, and oxygen consumption at 15 ^{o}C was about 80 mg O_2/kg/hr.).

This study also reported that white sturgeon, under hypoxic conditions were oxygen conformers. As oxygen tension decreased, gill ventilation frequency and heart rate remained steady, although the former dropped slightly under the most severe hypoxic stress. Branchial stroke volume declined with hypoxia, a strategy that may have served to reduce water flow through the gills, which would have increased residence time and, therefore, oxygen extraction. Routine oxygen consumption rate fell sharply with increasing hypoxia, reaching near negligible levels in very oxygen-poor water. Although data on other North American sturgeon species are scarce, Secor and Gunderson (1998) found that juvenile Atlantic sturgeon reduced their oxygen consumption rates from 250 to 440 mg O_2/kg/hr in normoxic water to 100 to 200 mg O_2/kg/hr under hypoxic conditions, indicating that this species, like white sturgeon, are oxygen conformers. Given that the Gulf subspecies is adapted for black water conditions that are prevalent in southern Louisiana and common throughout its range along the Gulf Coast, it is probable that this subspecies is an oxygen conformer as well.

5.1.4 Metabolism

In general, fish species from warmer waters have higher temperature preferences for growth. The ability of sturgeon to adapt to temperature change has only been examined in a few studies, some of which have been discussed previously. The optimal temperatures for growth and reproduction for small (small, 0.3 gm) juvenile Atlantic sturgeon (*Acipenser oxyrinchus*) as reported by Kelly and Arnold (1999) indicate optimal growth at 19 ^{o}C. The interactive effect of temperature and hypoxia indicate lower survival and growth at higher temperatures and low oxygen levels than at low temperature and hypoxia (Secor and Gunderson 1997), thus temperature may have a greater influence than hypoxia alone.

Streams in Louisiana's sub-tropical environment are characterized by warm temperatures, low gradients and water velocities, minimal re-aeration from riffles and high natural organic loads from riparian vegetation, all of which cause low (<5.0 mg/l) DO levels. It is very likely that hypoxic conditions (DO levels < 2.0 mg/l) have been a periodic if not pervasive condition that lotic fish assemblages in Louisiana have always been exposed to. Although anthropogenic activities can certainly exacerbate these conditions by affecting flow rates and organic loading, most Louisiana stream fishes can probably survive extended periods of low DO conditions (Felly 1992). Kelso et al. (2008) also noted that the critical questions for Louisiana stream systems revolve around the natural resistance and resilience of lotic coastal plain fishes to degraded water quality conditions (particularly low DO levels), which are a common phenomenon around the state (Ice and Sugden 2003).

Kelso et al. (2008) examined fish community composition and abundance as it relates to aquatic physicochemistry and habitat structure in least impacted streams in Louisiana that were hypoxic at the time of sampling and found no obvious differences in fish community composition relative to non-hypoxic systems. In general, Kelso et al. surmised that low DO conditions are not exceedingly problematic for fishes in lotic habitats in Louisiana given that these conditions occur naturally during the summer in stagnant or slow-flowing streams throughout the state that are bordered by abundant riparian vegetation. Kelso et al. did not find it surprising that hypoxic samples were not different in total fish abundance and taxonomic composition from samples taken from comparable non-hypoxic systems. This is not to say that pervasive hypoxia cannot be exacerbated by human activities that increase organic loadings and stream BOD, but Kelso et al. noted that hypoxic conditions may have to be chronic and severe (e.g., < 0.75 mg/L) to be reflected in fish community structure.

The most sensitive response has been reported by Burggren and Randall (1978), where movement-restrained white sturgeon (*Acipenser transmontanus*) exhibited reduced respiration at experimental DO_{SAT} conditions <90% (8.1 mg/L, 18 ºC). At the other extreme, Nonnotte et al. (1993) observed that Siberian sturgeon (*A. baeri*) maintained standard metabolism down to 25% DO_{SAT} (2.4 mg/L) at 15 ºC. The effects of DO_{SAT} on Atlantic sturgeon that Burggren and Randall (1978) observed were intermediate to these studies, and highly temperature-dependent. Niklitschek et al. observed similar metabolic rates at 70 and 100% DO_{SAT}, followed by a strong reduction in routine metabolism when DO saturation was lowered from 70% to 40%. Such saturation values are equivalent to DO concentrations of 5.24 and 2.99 mg/L, respectively, at 28 °C and salinity 8 ppt. Milder responses to low DO_{SAT} were observed as temperature decreased to 20 °C and, then, to 12 °C. Beyond possible species-specific differences (Taylor et al., 1999), discrepancy among previous results could be related to routine metabolism being more responsive to hypoxia than standard metabolism. In fact, a reduction in locomotor activity might be a primary reaction to hypoxia (Nilsson et al.., 1993; Crocker and Cech, 1997; Taylor et al.., 1999).

The Secor (1998) study was unique in examining the effects of long-term hypoxia on routine metabolism. As with many laboratory experiments in closed artificial conditions, Secor did not consider behaviors that can 1) reduce exposure to hypoxic waters and 2) compensate for reduced DO levels. Phil et al. (1991) and Brietburg (1992) have provided field evidence that fish will escape hypoxic conditions through local migrations. These behaviors include vertical or shoalward emigrations from hypoxic or anoxic bottom habitats. Following hypoxic events, bottom habitats are recolonized. Short-term episodic hypoxia may benefit bottom-feeding fish, given that burrowing macrobenthic prey will emerge at DO levels <2 mg/L, increasing their vulnerability to predation by fish that can tolerate short-term excursions into hypoxic waters (Phil et al., 1992). If unable to escape hypoxic conditions, sturgeon may be able to compensate by either surfacing to exploit higher oxygen concentrations in surface water or by adjusting their metabolic rate (e.g. through reduced swimming. (Cech et al., 1984). Secor et al. also noted that many fish in hypoxic environments move to the surface to relatively oxygen-rich water located at the air-water interface, and aerial respiration cannot be ruled out for sturgeons since they are physostomous.

Secor et al. noted that the Hudson River strain of Atlantic sturgeon that was used in their experiment might have exhibited a different response to hypoxia than a strain native to Chesapeake Bay. The Hudson River rarely becomes hypoxic (Cooper et al., 1988), and Atlantic sturgeon from the Hudson River may not have been adapted to hypoxic conditions. An aquaculture study by Serov et al. (1988) on stellate sturgeon *(Acipenser stellatus)* showed that heterozygosity in the LDH gene conferred survival advantages in hypoxic and high temperature conditions. Therefore, it is conceivable that Chesapeake Bay Atlantic sturgeon have adapted to hypoxic conditions over several generations. However, because generation time is extremely high in Atlantic sturgeon (c. a. 29 years [Stevenson and Secor, 1996]) and because hypoxia increased rapidly during this century in the Chesapeake Bay, Chesapeake Bay Atlantic sturgeon may not have been able to recoup historical abundances by dint of selection to low oxygen conditions.

The Secor et al. findings that varying strains of Atlantic sturgeon may have responded differently indicate a definable genetic difference exists between strains, and given that the Gulf sturgeon is a distinct subspecies of the Atlantic sturgeon, this is likely true of other strains as well. Campton et al. (2000) used mitochondrial DNA to examine genetic variation within and among three Pallid Sturgeon groups; two from the upper Missouri River and one from the Atchafalaya River. Although the Pallid Sturgeon from the upper Missouri River and Atchafalaya Rivers did not share any haplotypes, the genetic distance between these two groups (0.14%) was nearly as great as the genetic distance between Pallid and Shovelnose sturgeon in the upper Missouri River (0.15%). The authors note that this may represent reproductive isolation and genetic divergence between these two populations of Pallid sturgeon that is nearly as old as the isolation between Pallid and Shovelnose sturgeon. This suggests that Pallid sturgeon may have similar genetic adaptations to hypoxia and higher temperature typical of the black water environments that are found in the action area. Given the genetic variation separating the Atlantic sturgeon from the Gulf subspecies, it is likely that the Gulf sturgeon has a similar genetic adaption to black water environments that characterize the action area. (USFWS 2009).

Justus (2008) studied the natural biological setting of lowland streams in southwestern Louisiana in order to examine the value of using invertebrate and fish assemblages to investigate the ecological consequences of DO minima and provide information that can be used to help establish DO criteria for streams in southwestern Louisiana and other areas with coastal plains and large alluvial plains. Justus et al. did not develop metrics associated with intolerant taxa during their analysis because reference lowland streams (bayous that have little anthropogenic disturbance but are heavily forested and poorly flushed) can be expected to have DO conditions that are naturally limiting to almost all sensitive taxa (e.g. plecopterans). Given that there are few truly intolerant species (not to be confused with rare species) associated with lowland streams, intolerance metrics are not robust. Dissolved oxygen thresholds would be expected to be below DO criteria commonly established for the protection of aquatic life but well above the minimum DO concentration that is lethal to species native to lowland streams. The average DO thresholds determined for the invertebrate and fish assemblage (2.6 and 2.3 mg/L) slightly exceed DO criteria that are currently being applied to some coastal streams in Louisiana and Texas.

There are numerous references indicating that a large number of invertebrate and fish species are capable of tolerating DO concentrations of 1 mg/L (Moore, 1942; Doudoroff and Shumway, 1970; Davis, 1975; Kilgore and Hoover, 2001), which is slightly less than half of the average thresholds for these two assemblages. The time that fish can withstand low DO concentrations may depend on several factors (e.g. fish size, water temperature and behavior). Doudoroff and Shumway (1970) reported that some species (e.g. bluegill, (*Lepomis macrochirus*); orange spotted sunfish, (*Lepomis humilus*); warmouth, (*Lepomis gulosus*); and plains minnow, (*Hybognathus placitus*)) could tolerate DO concentrations around 1 mg/L for 18 h or longer when provided access to the surface, but survival was much lower when they could not access the surface.

Although DO minima generally had an inverse relation to the amount of agriculture in the buffer area, Justus (2008) reported that DO concentrations at three least disturbed sites with low amounts of agriculture also declined to less than 2.5 mg/L. Ice and Sugden (2003) found that in the summer, almost 60% of the forested least-impaired or reference streams in northern Louisiana had DO concentrations less than 3 mg/L. This indicates that in some lowland settings, the link between DO and degree of aeration and organic decomposition (i.e. flushing, Mallin et al., 2006) will sometimes be stronger than the link between DO and stream–nutrient concentrations. Although DO levels may fall to 2.5 mg/L or below, these concentrations are often the natural condition in Southern Louisiana blackwater streams, support aquatic communities that are uniquely adapted to these habitat conditions.

5.2 Response to DO in the Alabama heelsplitter

Unionoid mussels have a complex life cycle that makes them vulnerable to a wide variety of physical, chemical, and biological impacts. However, there have been limited studies on the limitation of respiration by hypoxia in adult unionoids (Sheldon and Walker 1989, Massabuau et al. 1991, Chen et al. 2001) or in early juveniles (Dimock and Wright 1993, Polhill and Dimock 1996).

Stern (1976) stated that like pH, the effects of low DO levels on freshwater mussels may be overstated in the literature. Ellis (1931) noted that mussels would not survive at oxygen levels below 5 mg/L, and Grantham (1969) found no living mussels where DO dropped as low as 3 mg/L. By contrast, Stern (1976) reported that live *Carunculina parva*, *Anodonta imbecilis*, and *Glebula rotundata* were collected from Bayou Manchac, LA in water with a mean DO content of only 2.6 mg/L along the bottom at a depth of 1 meter. Dietz (1974) demonstrated that *Ligumia subrostrata* is a facultative anaerobe and can survive for extended periods of time (greater than 15 days) without suffering an oxygen debt. This ability is of obvious ecological significance. Stern (1976) further noted that Louisiana drainages west of the Mississippi River are typically sluggish, turbid, and rich in organics, resulting in low DO levels for much of the year. Such habitats, however, often contain an abundant and diversified fauna. Finally, Stern (1976) also noted that two bayou species, *Plectomerus dombeyanus* and *G. rotundata*, show particular tolerance to low oxygen levels.

Barnhart et al. (2007 unpublished report) carried out a study focusing on the effects of hypoxia on immature life stages of selected species of freshwater mussels. Barnhart et al. examined the relationship between DO and rate of oxygen consumption (MO_2) of early juveniles of *Lampsilis siliquoidea* and *Lampsilis reeveiana*. Survival and growth of juveniles was tested in acute (4-day) and chronic (28-day) exposures to continuous hypoxia at 20, 25, and 30 °C. They also tested the effects of a daily cycle of hypoxia and air-saturation on survival and growth.

In addition to the juvenile life stage, the Barnhart study examined the survival of brooded glochidia larvae (marsupial) following 4-day and 28-day exposure of the brooding females to hypoxia. They also measured internal DO in the marsupial gill of brooding females, in order to compare DO in the ambient water and the marsupial space and test for compensatory changes in ventilation.

The respirometry results showed that the juveniles generally remained active throughout the respirometry measurements, with apertures open and crawling about the chamber, even when DO dropped to zero. Successive runs with the same group gave similar results, and MO_2 ($mg*kg^{-1}*h^{-1}$) increased with temperature. MO_2 of the brokenray (*Lampsilis reeveiana)* was higher than that of the fatmucket (*L. siliquoidea)* at 25 and 30 °C but not at 20 °C. The temperature sensitivity of MO_2 was higher at 20-25°C than at 25-30°C and was higher for brokenray than for fatmucket. Effects of continuous DO exposures on growth were inconsistent at these temperatures.

The Barnhart study also looked at survival of the 28-day intermittent (daily) hypoxia exposures. Survival exceeded 94% except at the lowest DO tested, 0.03 mg/L, where increased mortality was seen at day 12, and >50% mortality occurred by day 17. Mortalities were higher and increased sooner in the small and medium size classes than in the large size class. Brooding adult females of *Venustaconcha ellipsiformis* were tested in 4-day experiments. A 4-day exposure to a minimum DO of 0.03 mg/L did not increase mortality of females or brooded larvae. All adult females tested in 28-d experiments at a minimum DO 0.4 mg/L survived the exposures. Based on regression analysis, glochidia survival was reduced by 10% at 2.7 mg/L and by 50% at 1.7 mg/L in 28-day exposures.

DO in the marsupium was measured through numerous (8-14 h) recordings of brooding *Venustaconcha* and *Leptodea fragilis.* Both *Venustaconcha* and *Leptodea* ventilated during recording as indicated by open and extended apertures. DO in the marsupium (MDO) was always lower than in the outside water (WDO). During moderate ambient hypoxia, the ratio of MDO/WDO sometimes increased and MDO became more continuous, suggesting that ventilation became more continuous and partially compensated for hypoxia. However, when MDO was reduced to a low level, and particularly when MDO was at or near zero, raising WDO often did not immediately affect MDO, indicating that ventilation ceased at low WDO, and did not resume for as long as 30 minutes after WDO was elevated again. MDO sometimes fell to zero when WDO was still high. However, the results indicated that the shutdown of marsupial ventilation in fragile papershell (*L. fragilis*) did not always correlate with retraction of the apertures. The apertures sometimes remained extended and open, so that it appeared that water movement through the apertures continued, suggesting that ventilation of the marsupium in fragile papershell might be regulated independently of the respiratory ctenidium. This adaption may occur in other species as well.

Chen et al. (2001) examined the ability of adult freshwater unionoid species from different habitats to regulate oxygen metabolism under declining DO conditions. The effects of temperature were also evaluated for some species. The study was focused on the pattern of oxygen consumption changes under low DO rather than comparing the absolute values of specific oxygen consumption among different species. To quantify the ability of an animal to maintain oxygen consumption in low DO, Chen et al. used a hyperbolic model based on Bayne (1971) to provide a ratio as an index of respiratory independence from oxygen concentration.

The observed DO consumption values for varying species were related to their respective typical habitat type. *Villosa iris* and *V. constricta,* which generally live in well-oxygenated stream and river riffles, and *Pleurobema cordatum,* which occurs in areas of moderate flow and adequate oxygenation, exhibited the poorest ability to regulate oxygen consumption under conditions of low oxygen availability. *Pyganodon grandis, Amblema plicata, Quadrula pustulosa* and *Elliptio complanata*, which live in lentic habitats and lotic areas where DO typically declines in summer from algal blooms or organic decomposition, tend to generally have a greater ability to regulate oxygen consumption than the *Villosa* species. Byrne et al. (1995) also demonstrated that *E. complanata* tolerates a range of hypoxic conditions, even with zebra mussels *(Dreissena polymorpha)* attached to its valves. The species *E. fisheriana*, which lives in sand and is possibly exposed to hypoxia below the benthic surface, exhibited the greatest ability to regulate oxygen consumption.

The mechanisms of metabolic regulation in freshwater bivalves have not been studied extensively. The results from the Chen et al. (2001) study show some overlap in habitat and physiological ability to tolerate low DO in a given species, which seems to reflect differences that may be important biologically and agree with the limited published data for freshwater bivalves. For example, in a review of the ecology of freshwater mollusks, McMahon (1991) suggested that species living in aquatic habitats periodically subjected to hypoxia are better able to regulate oxygen consumption under declining DO conditions. Sheldon & Walker (1989) compared a species of mussel typical of flowing rivers with one found in impoundments that experience periods of low DO. The species from the river habitats *(Alathyria jacksoni)* was essentially a metabolic conformer, whereas the one from the impoundments *(Velesunio ambiguus)* exhibited metabolic regulation down to a partial pressure of 65 mm Hg. Hornbach (1991) observed that the freshwater clam *Musculium partumeium* shows excellent metabolic regulation down to a partial pressure of approximately 30 mm Hg. This species is commonly found in ephemeral ponds that experience frequent periods of low oxygen. Massabuau et al. (1991) reported that *Anodonta cygnea* (a freshwater bivalve) maintains oxygen consumption independent of ambient oxygen down to a low level primarily by maintaining arterial blood pressure at low values, independent of oxygen partial pressure in the water. Chen et al. (2001) noted that in a (unpublished) pilot study, when heart rate and oxygen consumption was monitored simultaneously in *Pyganodon grandis*, they found that these mussels increased their heart rate when DO was low, presumably to help maintain oxygen consumption, and it is possible that other species may use these and/or other mechanisms to facilitate metabolic regulation.

Both the Chen et al. (2001) results and others discussed indicate that species living in aquatic habitats periodically subjected to prolonged hypoxia may have a greater ability than those in other conditions to regulate oxygen consumption under declining DO. More specifically, the data presented in the Chen et al. (2001) study suggest water quality criteria for minimum DO at temperatures of around 24 °C represents a transition below which the mussels are not maintaining 'normal' oxygen consumption. Then at a DO below this transition, the animals may be under some degree of stress if the condition persisted for many hours or days (Davis, 1975). For those species that live in lentic habitats similar to but likely not as naturally low in DO as that of the heelsplitter (*Potamilius inflatus*), like *Amblema plicata, Quadrula pustulosa* and *Elliptio complanata*, this transition is around 2-3 mg/L^{-1} to ensure that aerobic metabolism remains relatively unchanged.

Although the specific DO tolerance range for the heelsplitter is the focus of this section, it is important to consider other factors that may be contributing to the decline of the heelsplitter in the action area. Landscape-scale variables affecting the distribution of threatened and endangered mussels have not been studied extensively. Among other threats common to unionoid mussels, heelsplitters in the Amite River are also threatened in the northern part of their range by gravel mining (Hartfield, 1993). Significant stretches of the Amite River have been subjected to extensive gravel mining since the 1950s, peaking in the 1980s, changing the Amite from a meandering river channel into a broader flood plain with a braided channel, eroded banks, and extensive headcutting (Hartfield, 1993; Mossa & McLean, 1997; Brim-box & Mossa, 1999), which may have had a significant effect on the distribution of this species. The more southern part of the species' range in the lower Amite River basin, from Baton Rouge to Lake Maurepas, currently is undergoing extensive urbanization from the growing Baton Rouge Metropolitan area directly west of the river.

Brown et al. (2010) hypothesized that the remaining populations of heelsplitters in the southern part of the Amite River are being affected by increased urbanization of the watershed, particularly by growth of the surrounding Baton Rouge metropolitan area. Brown et al. designed and carried out a study that concentrated on the remaining populations in the lower river, and included local factors along with reach-scale variables (e.g., land use) in a logistic regression to determine variables that successfully separated sites with and without heelsplitters. Their results were also compared with Brown & Curole (1997) to determine if the catch per unit effort (CPUE) and the adult size distribution of heelsplitters changed since 1994.

The authors quantified predictor variables at four spatial scales. Site variables included substrate composition (percentage sand, silt or gravel), DO, water temperature, specific conductivity, current velocity, and channel wetted river width. They used Louisiana Gap Analysis Project (LAGAP) digital spatial data describing vegetation coverage and land use at 1:100,000 during 1992 (LAGAP, 2000). Maps were developed from satellite imagery, botanical surveys, aerial photography, and existing coastal Louisiana habitat maps, to describe land use/land cover in 30 m pixels with classes developed by the Louisiana Natural Heritage Program (LAGAP, 2000). The resulting comparison of CPUE in 2007 with a study conducted in 1994 indicated a significant drop in CPUE from 1.76 heelsplitters per site to 0.87. The size distribution of heelsplitters also had decreased in mean shell length from 116 to 97 mm, owing either to dislodgement of larger individuals in spates, or die-offs of larger males.

The logistic regression suggested that site variables like substrate type and current velocity were not as important as landscape-scale variables in predicting heelsplitter presence at a site. Heelsplitter presence was positively related to the amount of wetland riparian forest, and negatively related to the amount of residential development at the reach (1 km upstream) scale. These results are significant because they show that site variables such as substrate composition (percentage sand, silt or gravel), DO, water temperature and specific conductivity are not as significant as the integrity of the riparian corridor to the survival and recovery of heelsplitter populations.

The level of metabolic regulation of oxygen concentration in waters with reduced DO levels has been widely investigated for marine species of bivalves from a range of habitats (e.g. Bayne. 1971; Taylor & Brand, 1975; Shumway & Koehn, 1982; Wang & Widdows 1993). The studies discussed here indicate that the extent of metabolic regulation varies with the environmental conditions, body size, physiological state of the animals, and most especially, the habitat where the species is generally found.

6.0 Analysis of Effects

EPA's task through this BE is to consider the ecophysiological response to stressors, specifically DO, in an effort to determine if a potential action for EPA to approve the minimum 2.3 mg/L criterion would adversely affect the species of interest dependent on water column DO for respiration. The listed species of interest potentially affected by the proposed action include the Atlantic sturgeon (Gulf subspecies) (*Acipenser oxyrinchus*) (=*oxyrhynchus desotoi*), the Pallid sturgeon (*Scaphirhynchus albus*) and the Alabama (=inflated) heelsplitter (*Potamilus inflatus*).

Louisiana, similar to a number of other states, has addressed revising water quality criteria on a site-specific basis. Using this approach, Louisiana established a minimum DO criterion of 2.3 mg/L that applies between the months of March to November for 31 inland freshwater and estuarine stream subsegments in the eastern LMRAP. This DO criterion does not apply between the months of December to February; a one-day minimum criterion of 5.0 mg/L in inland areas and 4.0 mg/L in estuarine areas continues to apply during the three months of winter except where site-specific criteria have been established. The EPA's analysis assumes that listed species of interest are exposed to waters meeting water quality standards. The only action under consideration at this time is whether the revised DO standard itself and EPA's approval of it will have an effect on the species of interest. Dissolved oxygen levels will vary during normal diurnal fluctuations typically seen in black water habitats in southern Louisiana.

Scientific information available for threatened and endangered species is often limited. In evaluating potential effects, EPA considered a number of cited sources that used surrogate species/strains and experimental designs that place subject animal models to hypoxic or other adverse conditions for extended periods that are unlikely to occur under natural conditions to illicit a physiological response. Although specific to sturgeons, Niklitshek et al (2009) said that the results of such experimental designs could be interpreted as meaning that sensitivity to hypoxia is higher than in other fishes, such as rainbow trout (*Oncorynchus mykiss*). Niklitshek also noted that this overall pattern of especially high sensitivity of sturgeons to hypoxia seems counterintuitive since many sturgeon populations may have historically used shallow warm estuaries, where hypoxia occurred naturally in the deepest waters (Burggren and Randall, 1978; Crocker and Cech, 1997). These designs tend to create conditions that limit behavioral responses to stress that the subject animals would typically be able to avoid in natural settings.

6.1 Final Effect Determination for Listed Species

6.1.1 Gulf (*Acipenser oxyrhynchus desotoi*)

The cited studies discussed previously describe various sturgeon species' physiological response to stressors for significant periods of time in controlled environments that limit or prevent critical behavioral responses. The data indicate that sturgeon species in general have metabolic and behavioral responses to low DO conditions. Atlantic sturgeons show a partial shift to anaerobic metabolism under low DO conditions. The Gulf sturgeon is an anadromous fish, with populations in the western Gulf of Mexico inhabiting low gradient black water streams to coastal Gulf waters. The Gulf sturgeon evolved in and is adapted to stream conditions that are naturally low velocity, with mud, clay, and silt bottoms and high biomass limiting DO levels. The Gulf subspecies' genetic makeup likely provides a greater ability to shift its metabolism in anaerobic conditions. In addition to these metabolic adaptations, Gulf sturgeon exhibit behavioral responses to DO stress common to any fish species.

Critical Habitat

The Gulf sturgeons like other sturgeons, are a long-lived, late-maturing, intermittent spawning species. These life cycle characteristics make the Gulf sturgeon vulnerable to a number of threats. In its Gulf sturgeon Recovery Plan and 5-Year Review (USFWS 1995, 2009), the USFWS noted that within some drainages, anthropogenic physical alterations such as dams, diversions, and dredging may affect access to historical habitat, disproportionately impacting spawning and juvenile life stages. Other potential anthropogenic factors climate change, red tide, by-catch, collisions with boats, point source and nonpoint source discharges.

Part of EPA's task in this BE is to evaluate if the approval of the minimum 2.3 mg/L criterion would adversely affect critical habitat for the Gulf sturgeon. There are no direct effects to critical habitat as a result of EPA's approval of Louisiana's revised DO criteria. As discussed previously, approving new water quality standards in and of itself will not change the environmental baseline or directly affect listed species or species proposed for listing. Potential indirect effects may exist because the approval allows implementation of the revised DO criteria. This includes NPDES permits, 303(d) assessment and listings, development of TMDLs, and water quality management plans. However, these indirect effects will not result in physical alterations such as dams, diversions, or dredging that may affect access to historical habitat.

Based on these findings, EPA has determined that the approval of Louisiana's minimum DO criterion of 2.3 mg/L is not likely to adversely affect (NLAA) the Gulf sturgeon.

6.1.2 Pallid sturgeon (*Scaphirhynchus albus*)

As discussed above, the cited studies describe physiological response to stressors for significant periods of time in controlled environments that limit or prevent critical behavioral responses in various sturgeon species. The data indicating that sturgeon species in general have metabolic and behavioral responses to low DO conditions is significant.

The cited literature suggests that like the Gulf sturgeon, the diverse habitats of the Pallid sturgeon have likely lead to significant genetic variation. As noted in the species description, there are indications that the northern and southern Pallid sturgeon arose independently from different ancestors and represent two separate species (Campton et al. 2000), These data (Campton et al. 2000, Tranah et al. 2001, Heist and Schrey 2006a) suggest that the genetic structuring within the Pallid sturgeon's range represents two distinct groups at the extremes of the species range. This pattern is suggestive of a pattern of isolation by distance, with gene flow more likely to occur between adjacent groups, and thus, genetic differences in those in the southern geographical range providing a greater ability to shift its metabolism in anaerobic conditions. In addition to these metabolic adaptations, Pallid sturgeon also exhibit behavioral responses to DO stress common to any fish species.

Based on these findings, EPA has determined that the approval of Louisiana's one-day minimum DO criterion of 2.3 mg/L is not likely to adversely affect (NLAA) the Pallid sturgeon.

6.1.3 Alabama heelsplitter (*Potamilius inflatus*)

EPA considered the available information in the literature, looking primarily at how low DO may affect the Alabama heelsplitter. As noted in the discussion of the heelsplitter (Bayne. 1971; Taylor & Brand, 1975; Shumway & Koehn, 1982; Wang & Widdows 1993), studies indicate that the extent of metabolic regulation varies with the environmental conditions, body size, physiological state of the animals, and most especially, the habitat where the species is generally found. A notable finding was that like pH, the effects of low DO levels on freshwater mussels may be overstated in the literature.

The results from Chen et al. (2001) suggested that water temperature rather than DO concentration itself represents a transition below which mussels are less able to maintain 'normal' oxygen consumption as opposed to DO levels per se. Chen et al. also noted that DO consumption values for varying species were related to their respective typical habitat type. Species that generally live in well-oxygenated habitats exhibited the poorest ability to regulate oxygen consumption under conditions of low oxygen availability. Thus the heelsplitter, which occurs in aquatic habitats periodically subjected to prolonged hypoxia, may have a greater ability than those in less variable conditions to regulate oxygen in conditions of declining DO.

Based on these findings, EPA has determined that the approval of Louisiana's one-day minimum DO criterion of 2.3 mg/L is not likely to adversely affect (NLAA) the Alabama heelsplitter.

References

Barnhart, C. and Kaiser, B. (2007). *Effects of Hypoxia on Freshwater Mussels*. Missouri State University, 901 S. National, Springfield, MO 65897. Unpublished final report.

Brown, K. M., George, G. and Daniel, W. (2010) Urbanization and a threatened freshwater mussel: evidence from landscape scale studies. *Hydrobiologia*. 655:189–196 DOI 10.1007/s10750-010-0448-8.

Cech, J. Jr., Dorshov, S. I., (2005). Environmental Requirements, Preferences and Tolerance Limits of North American Sturgeons in: G. LeBreton, F. Beamish and R. McKinley, ed., *Sturgeon and paddlefish of North America*. Dordrecht: Kluwer Academic Publishers, pp. 73-86.

Chen, L., Heath, A. G., Neves, R. J. (2001). Comparison of oxygen consumption in freshwater mussels (Unionidae) from different habitats during declining DO concentration. *Hydrobiologia* . 45: 209-214. Kluwer Academic Publishers, Dordrecht, NL.

Crocker, C. E., and Cech. J. J. Jr. (1997). Effects of environmental hypoxia on oxygen consumption rate and swimming activity in juvenile white sturgeon, *Acipenser transmontanus*, in relation to temperature and life intervals. *Environmental Biology of Fishes* **50**: 383–389, 1997. Kluwer Academic Publishers, Dordrecht, NL.

DeWalt, E., (1995). *Biological communities of reference streams in the South Central Plains and Upper Mississippi Alluvial Plains Ecoregions*. Louisiana Department of Environmental Quality, Office of Water Resources. Baton Rouge, Louisiana.

DeWalt, E., (1997). *Fish and macroinvertebrate taxa richness, habitat quality, and in-situ water chemistry of ecoregion reference streams in the Western Gulf Coastal Plains and Terrace Upland Ecoregions of southern Louisiana*. Louisiana Department of Environmental Quality, Office of Water Resources. Baton Rouge, Louisiana.

Justus, B. G., Mize, S. V., Wallace, J. and Kroes, D. (2012). Invertebrate and Fish Assemblage Relations to Dissolved Oxygen Minima in Lowland Streams of Southwestern Louisiana. *River Research and Applications*. Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/rra.2623.

Kelso, W. E. D., Rutherford, A. and Kaller. M. D. (2008). *Relaionships Between Nutrients, Dissolved Oxygen Conditions*, *Habitat and Fish Assemblage Composition in Louisiana Streams*. Final Report Prepared for the Louisiana Department of Environmental Quality.

LeBreton, G. T., Beamish, F. W. and McKinley, R. S. (2005). *Sturgeon and paddlefish of North America*. Kluwer Academic Publishers, Dordrecht, NL.

Louisiana Department of Environmental Quality, (1996). *Quality Assurance Plan for Louisiana Ecoregion Project*. Office of Environmental Assessment, Water Quality Assessment Division. Baton Rouge, LA. 104(b) Grant X-006647-01.

Niklitschek, E. J. and Secor, D. H. (1997). DO, temperature and salinity effects on the ecophysiology and survival of juvenile Atlantic sturgeon in estuarine waters: I. Laboratory results. *Journal of Experimental Marine Biology and Ecology* 381: S150–S160.

Peake, S. J. (2005). Swimming and Respiration in: G. LeBreton, F. Beamish and R. McKinley, ed., *Sturgeon and paddlefish of North America*. Dordrecht: Kluwer Academic Publishers, pp. 147-166.

Secor, D. H. and Gunderson, T. E. (1998). Effects of hypoxia and temperature on survival, growth, and respiration of juvenile Atlantic sturgeon, *Acipenser oxyrinchus*. Fishery Bulletin 96:603-613.

Singer, T. D., Ballantyne, J. S., Sturgeon and Paddlefish Metabolism in: G. LeBreton, F. Beamish and R. McKinley, ed., *Sturgeon and paddlefish of North America*.: Kluwer Academic Publishers, Dordrecht, NL. pp. 167-194.

Smythe, D., (1999). *Overview of the 1995 and 1996 Reference Streams*. Louisiana Department of Environmental Quality, Office of Environmental Assessment, Environmental Technology Division. Baton Rouge, Louisiana.

Smythe-Genovese, D., (2001). *Overview of the 1995 Through 1998 Reference Streams*. Louisiana Department of Environmental Quality, Office of Environmental Assessment, Environmental Technology Division. Baton Rouge, LA.

Stern, E. M. (1976). *The Freshwater Mussels (Unionidae) of the Lake Maurepas - Pontchartrain -Borgne Drainage System, Louisiana and Mississippi*. LSU Historical Dissertations and Theses. 2944. http://digitalcommons.lsu.edu/gradschool_disstheses/2944.

U.S. Environmental Protection Agency (2009). National recommended water quality criteria. Offices of Water and Science and Technology. Washington, DC.

U.S. Fish and Wildlife Service. 2014. Revised RECOVERY PLAN for the Pallid Sturgeon (*Scaphirhynchus albus*). U.S. Fish and Wildlife Service. Billings, Montana.

Wilson, J. A., McKinley, S. (2005). Distribution, Habitat and Movements in: G. LeBreton, F. Beamish and R. McKinley, ed., *Sturgeon and paddlefish of North America*. Kluwer Academic Publishers, Dordrecht, NL. pp. 40-72.

Appendix A

Appendix B